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DETERMINATION AND PREDICTION OF PRODUCTS OF TRACE METAL REMOVAL IN LABORATORY AND FIELD SCALE VERTICAL FLOW BIOREACTORS

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DETERMINATION AND PREDICTION OF PRODUCTS OF TRACE METAL REMOVAL IN LABORATORY AND FIELD SCALE VERTICAL FLOW BIOREACTORS

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SCHOOL OF CIVIL ENGINEERING AND ENVIRONMENTAL SCIENCE

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Abstract

Mine drainage is characterized by elevated concentrations of dissolved iron, sulfate, and trace metals and generally exhibits an acidic pH. These constituents, particularly trace metals, can pose serious risks to both environmental and human health and require some sort of treatment. Passive treatment systems (PTS) have become a common technology used in the treatment of mine drainage. A component of these systems, vertical flow bioreactors (VFBR), may be used to remove trace metals through a variety of mechanisms, resulting in different removal products.

Ionic strength is a measure of dissolved ions in solution and correlates strongly with total dissolved solids (TDS) concentrations and conductivity. In mine drainages, ionic strength is commonly dominated by the sulfate anion and various cations, including calcium, magnesium, iron, and occasionally sodium. Large concentrations of these constituents may impact trace metal removal processes in VFBR, including sulfide precipitation, adsorption to and complexation with organic matter, and carbonate precipitation by impacting solubility and availability.

The premises of this dissertation are that trace metals are removed from mine drainage through a variety of mechanisms in vertical flow bioreactors, that ionic strength has an impact on which mechanisms play a dominant role in trace metal removal, and that geochemical modeling can be used to predict how trace metals will be retained in VFBR. Three studies, resulting in four chapters, were performed to evaluate these ideas.

Chapters One and Two describe the results of the first study, which evaluated ionic strength impacts on trace metal removal in VFBR. Chapter One, “Evaluation of the impact of Na-SO_4 dominated ionic strength on effluent water quality in bench-scale vertical flow bioreactors using spent mushroom compost” specifically addresses the effects on water quality as a result of elevated ionic strength, whereas Chapter Two, “Evaluating the impact of Na-SO_4 dominated ionic strength on trace metal removal products in bench-scale vertical flow bioreactors using sequential extractions, acid-volatile sulfide analyses, and mineralogical methods” addresses the effects of ionic strength on the products of trace metal removal. Elevated ionic strength appeared to have significant impacts on pH, alkalinity, and trace metal removal. Elevated ionic strength had differing effects on the five trace metals examined, with an increase in ionic strength increasing the rate of removal of Cd and Zn and decreasing the rate of removal of Ni and Mn. Despite the differences observed in effluent concentrations between different ionic strengths, there were no significant differences in total metals concentrations in the associated substrates. Elevated ionic strength resulted in less Cd being retained as an insoluble sulfide, while the opposite was true for Pb. In addition, increased ionic strength caused a decrease in the amount of Pb retained in the labile organic fraction. The ratio of simultaneously extracted metals to acid-volatile sulfides (SEM/AVS) indicated that a large portion of Cd, Mn, Ni, Pb, Zn was likely in soluble and bioavailable forms. When all of the removal products are considered, Ni retention within the substrate was the most stable and Mn was the least stable of the trace metals examined.

Chapter Three, “Characterization of mine drainage vertical flow bioreactor substrates using sequential extractions, acid-volatile sulfide analyses, and scanning electron microscopy,” examined products of trace metal removal in a field-scale VFBR. Water quality data collected at a VFBR in the Tar Creek Superfund Site in northeastern Oklahoma indicate significant removal of trace metals from the mine drainage. Spent substrate samples from the VFBR were collected after 5.5 years of system operation. Results of a sequential extraction procedure (SEP) showed that the vast majority of Cd, Co, Fe, Ni, Pb, and Zn were retained in the VFBR as insoluble sulfides. Subsequent acid-volatile sulfide/simultaneously extracted metals (AVS-SEM) analyses confirmed the retention of Cd, Fe, Pb, and Zn as sulfides, but Co and Ni results were less certain due to the lack of solubility of Co- and Ni-sulfides in cold, dilute hydrochloric acid. With the exception of Mn, trace metals were retained as insoluble products in the VFBR.

Results presented in Chapters One, Two, and Three were used to perform a geochemical modeling study, the results of which are described in Chapter Four, “Using reactive transport modeling to predict trace metal removal products in vertical flow bioreactors.” Water quality data and SEP results from the experimental VFBR described in the first two chapters were used to construct and calibrate a reactive transport model in PHREEQC, which was then validated with data from the third chapter and samples from a third party. The model results provided fair predictions of VFBR effluent quality. Predictions of trace metal removal products were upheld by SEP results, with the exception of the labile organic fraction, which was generally underpredicted.

Chapter One: Evaluation of the impact of Na-SO_4 dominated ionic strength on effluent water quality in bench-scale vertical flow bioreactors using spent mushroom compost

This chapter has been formatted and submitted to *Mine Water and the Environment*.

Abstract

Passive treatment systems (PTS) are frequently used to treat mine drainages containing elevated concentrations of Fe, sulfate, and trace metals. Vertical flow bioreactors (VFBR), often designed specifically to remove trace metals from mine drainage and retain them in an organic substrate, are a common component of PTS. Many factors may impact the performance of VFBR, including ionic strength, thereby affecting the ability of the systems to remove trace metals. A paired-comparison study was performed in which two sets of columns were constructed, filled with an organic substrate, and fed synthetic mine drainage of differing ionic strengths, dominated by sodium and sulfate, for one year. Effluent samples were collected and analyzed for Cd, Mn, Ni, Pb, and Zn every two weeks. Differences in ionic strength had variable effects on the five metals examined. Elevated ionic strength significantly increased the rate of removal of Cd and Zn, and possibly Pb. Conversely, elevated ionic strength significantly decreased the rate of removal of Ni and Mn and even caused the eventual release of Mn from the substrate. The processes by which trace metals were removed from solution and retained in the organic substrate were not evaluated in this study, but a subsequent study on the spent substrates will elucidate more clearly the impact of elevated ionic strength on products of trace metal removal in VFBR.

Introduction

Mine drainage is characterized by elevated cations (e.g., Fe^{2+}, Na^{+}, Ca^{2+}, Mg^{2+}) and sulfate (SO_4^{2-}) concentrations, produced by the oxidation of pyrite and other sulfide minerals and through secondary reactions and ion exchange processes (Capo et al. 2001; Younger et al. 2002; Watzlaf et al. 2004). Base cations (Na^{+}, Ca^{2+}, Mg^{2+}) and sulfate, as well as bicarbonate (HCO_3^{-}) alkalinity, make up the majority of the inorganic contribution to total dissolved solids (TDS) in mine drainages (Skousen et al. 2000; Palmer et al. 2010, Timpano et al. 2010). Total dissolved solids, conductivity, and ionic strength are closely related parameters, with all three representing the sum total of all ions in a solution (Hem 1985, Cravotta 2008, Cravotta and Brady 2015).

Vertical flow bioreactors (VFBR) were originally conceived as a method to create anoxic, reducing conditions to promote alkalinity generation via bacterial sulfate reduction (BSR) and dissolution of limestone mixed with or underlying an organic substrate (Kepler and McCleary 1994). However, for hard rock mine drainage, VFBR have also been used extensively to remove trace metals through sorption to organic matter and through precipitation as sulfides, hydroxides, and carbonates (Dvorak et al. 1992, Machemer and Wildeman 1992, Webb et al. 1998, Neculita et al. 2008). In addition, VFBR can be very efficient at simply removing large amounts of sulfate, depending upon substrate used and hydraulic retention time (Dvorak et al. 1992, Cocos et al. 2002, Willow and Cohen 2003, Neculita et al. 2007, Pinto et al. 2015).

When treating net-alkaline hard rock mine drainages, VFBR will often be preceded by oxidation and settling ponds, where Fe^{2+} is oxidized to Fe^{3+}, hydrolyzed, and precipitated as Fe oxyhydroxides (e.g. FeOOH) (Watzlaf et al. 2004). As a result, water entering VFBR will often exhibit near-neutral pH, but will have lost a substantial amount of alkalinity (Dvorak et al. 1992, Neculita et al. 2007). Although small amounts of trace metals may be removed via sorption and coprecipitation with Fe oxyhydroxides, the remainder may be removed in VFBR (Dzombak and Morel 1990, Kairies et al 2005). In addition, some sulfate may be removed via sorption to Fe oxyhydroxides, but the majority will make its way to the VFBR (Ali and Dzombak 1996, Webster et al. 1998).

Although secondary standards for drinking water already exist, the United States Environmental Protection Agency has proposed protocols for regulating TDS concentrations in natural waters (USEPA 2011). However, TDS removal down to the proposed limits via conventional mine drainage treatment schemes can be inconsistent (Pinto et al. 2015). In addition, elevated TDS concentrations may impact some methods of treatment (Hem 1985, Langmuir 1997, Cravotta 2008).

Elevated ionic strength may work to enhance or inhibit trace metal removal in VFBR. An increase in bicarbonate concentration and pH due to bacterial sulfate reduction may provide a suitable environment for divalent trace metals to precipitate as carbonates (Dvorak et al. 1992, Sobolewski 1996). However, some studies have shown an increase in metal-carbonate solubilities as a result of increased ionic strength, leaving trace metals to be removed by other mechanisms or left in solution (Plassard et al. 2000, Sun et al. 2009). Theoretically, sulfide mineral solubility will also increase as ionic strength increases (Lewis 2010). However, at least one study has found that the solubility of some sulfide minerals may decrease as ionic strength increases (Shpiner et al. 2009). In addition, ionic strength may impact the solubility of hydrogen sulfide, thus limiting the potential for metal-sulfide formation (Duan et al. 2007). In order to evaluate the impact of TDS/conductivity/ionic strength on trace metal removal efficiency, an examination of how they affect VFBR performance as a whole is necessary.

Experimental design

Column construction

To evaluate the impact of ionic strength on trace metal removal in vertical flow bioreactors, a paired comparison column study was designed. Continuously fed, downward-flow columns were used to approximate typical field bioreactors. A hydraulic retention time (HRT) of 72 hours was used in order to provide ample time for sulfate reduction and subsequent sulfide precipitation (Neculita et al. 2007). In addition to HRT, a target flow rate of approximately three L/day and an assumed substrate porosity of 0.5 were used to determine the dimensions of the columns. These calculations resulted in the construction of six-38 cm (15 in) long columns constructed with 25 cm (10 in) diameter PVC pipe, each with a total volume of 19.3 L. PVC caps with 1.3 cm (0.5 in) threaded holes were used to enclose the columns. Opaque PVC was used in order to better simulate field bioreactor conditions and to prevent the growth of phototrophic bacteria and algae.

The effluent port was covered with 20-mesh nylon screen, which was covered with a 2.5 cm (1 in) layer of inert pea gravel, in order to prevent large particulates from entering and collecting in the effluent tubing. Over the pea gravel, all columns were filled with a 2:1 mixture (by volume) of spent mushroom compost (SMC) and inert river rock (to provide structure and prevent compaction/loss of permeability). Readily available SMC from J-M Farms, Inc. in Miami, OK was used as the substrate and carbon source. This source of SMC is made up of a base of wheat straw amended with chicken litter, cottonseed meal, soybean meal, peat moss, sugar beet lime, and gypsum. Water elevation was controlled by maintaining the effluent tubing at an elevation approximately 2 cm below the top of the columns. An illustration of the column setup is provided in Figure 1.1.

Simulated mine drainage

The six columns were divided into two sets of three replicates; one set receiving “low” ionic strength (LI) simulated mine drainage (SMD) and the other receiving “high” ionic strength (HI) SMD. All SMD was intended to simulate approximate conditions in net-alkaline mine drainage after Fe has been removed in an oxidation pond. Both LI SMD and HI SMD contained 0.5 mg/L each of Cd, Mn, Ni, Pb, and Zn. However, the LI SMD contained base cation concentrations of 10 mg/L Ca and 25 mg/L Na and anion concentrations of 15 mg/L Cl and 125 mg/L sulfate, resulting in an approximate ionic strength of 10^{-3} M. In contrast, the HI SMD contained base cation concentrations of 250 mg/L Ca and 1000 mg/L Na and anion concentrations of 440 mg/L Cl and 2100 mg/L sulfate, resulting in an approximate ionic strength of 10^{-1} M.

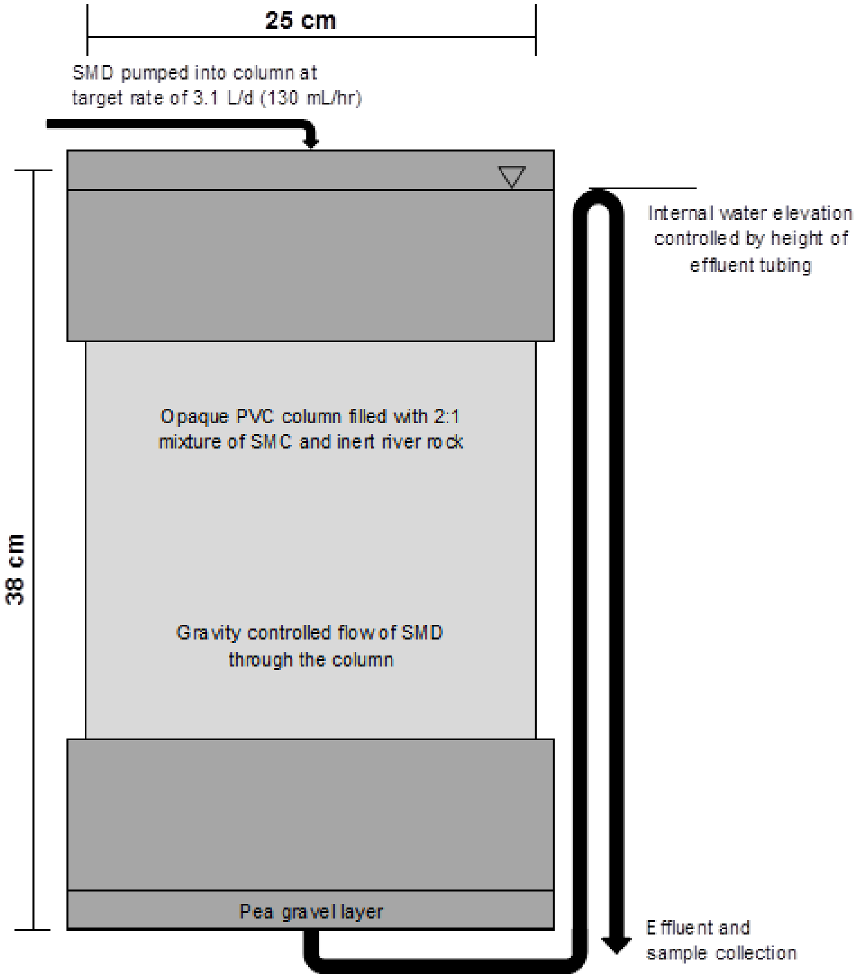


Figure 1.1 Schematic of experimental setup

Each SMD was prepared in a 50-L carboy approximately every five days. The respective SMD solutions were fed into the top of each column with peristaltic pumps at a target flow rate of 3.1 L/d (130 mL/hour) and allowed to flow naturally via gravity through the remainder of the system.

Methods

After an adjustment period of four weeks, effluent samples were collected from each column every two weeks for one year (approximately equivalent to 100 pore volumes). Temperature, pH, dissolved oxygen, conductivity, oxidation-reduction potential, and total alkalinity were measured immediately upon sample collection. Samples for total and dissolved metals analyses were collected in 60-mL HDPE bottles and acidified with trace metal grade nitric acid to $pH < 2$. Dissolved metals samples were filtered with 0.45-μm nylon syringe filters prior to acidification. Samples for total and dissolved carbon analyses were collected in 40-mL amber glass bottles that had been pre-baked at 400° C for one hour. Dissolved carbon samples were filtered with 0.45 μm nylon syringe filters and all carbon samples were stored at ≤ 4° C and analyzed as soon as possible after collection. Samples for sulfate and chloride analyses were collected in 30-mL polypropylene scintillation vials, stored at ≤ 4° C, and analyzed within 48 hours. Sulfide samples were collected in 60-mL HDPE bottles, preserved with zinc acetate and sodium hydroxide, and analyzed within 24 hours. Methods used for all sample analyses are listed in Table 1.1.

In addition to samples collected for chemical analyses, effluent samples were collected quarterly for determination of SRB populations, as well as overall anaerobic bacteria populations. Bacteria culture bottles containing either the American Petroleum Institute RP-38 sulfate reducer medium (for SRB) or thioglycollate medium (for anaerobic bacteria) were obtained from VK Enterprises in Edmond, OK. Bottles were inoculated and serial diluted to 10^{-6} to determine populations in both media. Samples were incubated at room temperature up to 28 days before determining the populations.

Table 1.1 Parameters measured throughout the study and the instrumentation and methods used to perform the analyses

Parameter	Instrumentation	Method/Source
Temperature, pH, DO, conductivity, ORP	Accumet XL600 benchtop multimeter	
Total alkalinity	Digital titrator	Hach 8203
Total and dissolved metals	CEM MARS Xpress digestion system	EPA 3015
	Varian Vista-PRO simultaneous axial ICP-OES	EPA 6010C
Total and dissolved carbon	Analytik Jena N/C 2100	EPA 9060A
Sulfate	Hach DR3800 spectrophotometer	Hach 8051
Sulfide	Spectrophotometer	APHA 4500 D

Statistics

The statistical software package Minitab version 17.2.1was used to compute all statistics (Minitab 2010). A significance level of 0.05 was applied. All data sets were checked for normality using the Anderson-Darling statistic. The Mann-Whitney test (also known as the Wilcoxen rank sum test) was used to determine significant difference between two sets of data. Upward and/or downward trends were determined using the Mann-Kendall test.

Results and Discussion

Influent characteristics

The LI and HI columns were each fed SMD by their own low-flow peristaltic pump. Over the course of the study, an average flow of 3.24 L/d (2.25 L/min) was maintained in the LI columns and an average flow of 3.11 L/d (2.16 mL/min) was maintained in the HI columns. Seventy batches (50-L carboys) each of LI SMD and HI SMD were prepared throughout the study and each column treated approximately 1215 L of SMD, which took 375 days for the LI set of columns and 391 days for the HI set of columns.

LI SMD and HI SMD samples were collected directly from their carboys approximately every ten batches throughout the study. Mean SMD water quality is listed in Table 1.2. Sulfide data are not included in the table as all samples contained the constituent at concentrations below the detection limit of 0.1 mg/L. In addition, all total carbon concentrations were below 1.0 mg/L and have been excluded from the table. Mean total and dissolved metals data are listed in Table 1.3.

There were significant differences in conductivity, pH, and sulfate between the LI SMD and HI SMD. The differences in conductivity and sulfate were due to the designed differences in chemical constituents and ionic strength and were expected. The difference in pH was most likely due to the difference in sulfate concentrations. An increased concentration in sulfate was correlated with a lower pH, as indicated by the Spearman’s rho value of -0.705 ($p = 0.023$). Cravotta

(2008) found a similar correlation between pH and sulfate in a survey of 140 coal mine drainages in Pennsylvania. Although there was a significant difference in pH in LI SMD and HI SMD, the values were within the desirable range for treatment in a VFBR.

Table 1.2 Mean influent water quality data and standard deviations

	LI SMD (n = 8)		HI SMD (n = 8)	
	Mean	**SD**	**Mean**	**SD**
Temperature (°C)	21.08	0.54	20.78	0.64
Conductivity (mS/cm)	0.32	0.01	4.91	0.10
pH (s.u.)	6.32	0.11	5.95	0.03
Dissolved Oxygen (mg/L)	8.05	0.45	8.32	0.20
Oxidation-reduction potential (mV)	213.50	14.54	185.60	38.93
Alkalinity (mg/L as $CaCO_3$)	5.70	2.97	3.15	0.32
Sulfate (mg/L)	103.60	1.52	1827	53.50
Chloride (mg/L)	24.00	0.27	425.77	6.99

Table 1.3 Mean influent metals concentrations and standard deviations

	LI SMD (n = 8)				HI SMD (n = 8)			
	Total		**Dissolved**		**Total**		**Dissolved**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Cd (mg/L)	0.48	0.01	0.48	0.01	0.48	0.02	0.48	0.01
Mn (mg/L)	0.49	0.02	0.49	0.02	0.46	0.01	0.46	0.01
Ni (mg/L)	0.48	0.01	0.48	0.01	0.45	0.01	0.45	0.02
Pb (mg/L)	0.46	0.04	0.44	0.08	0.59	0.10	0.58	0.09
Zn (mg/L)	0.46	0.01	0.46	0.01	0.49	0.03	0.49	0.02
Ca (mg/L)	37.43	1.05	38.56	1.36	266.58	13.77	259.50	12.88
Na (mg/L)	15.55	0.44	15.65	0.23	768.66	35.38	707.29	31.34

Total and dissolved Pb concentrations in HI SMD were closer to the target 0.5 mg/L in the first two batches at the beginning of the study. However, a new supply of Na_2SO_4 was obtained and inadvertently contributed an additional source of Pb to the HI SMD. This disparity accounts for the significant difference in LI and HI SMD Pb concentrations and larger standard deviations in Pb concentrations in both

SMD. However, there were no significant differences in mean Cd, Mn, Ni, and Zn concentrations between LI SMD and HI SMD. A significant difference was also found between the total and dissolved Na concentrations in the HI SMD, most likely due to incomplete dissolution of the Na_2SO_4 salt used to prepare the solution.

Effluent characteristics

Mean effluent water quality data are shown in Table 1.4. Over the course of the study, all columns sustained anoxic and reducing conditions and exhibited near-neutral pH. Dissolved oxygen concentrations quickly dropped to < 1 mg/L and remained so throughout the study. In addition, reducing conditions developed in all columns within two weeks after saturation and oxidation-reduction potential remained below -200 mV in LI and HI column effluents for the duration of the study. Chloride was conservative through both sets of columns, with no significant change in concentrations.

Table 1.4 Mean effluent water quality data and standard deviations

	LI effluent (n = 22)		**HI effluent (n = 22)**	
	Mean	**SD**	**Mean**	**SD**
Temperature (°C)	20.61	0.34	20.56	0.40
Conductivity (mS/cm)	0.57	0.40	4.88	0.22
pH (s.u.)	7.37	0.18	7.62	0.30
Dissolved Oxygen (mg/L)	0.21	0.34	0.04	0.09
Oxidation-reduction potential (mV)	-258.61	70.66	-314.08	33.84
Alkalinity (mg/L as $CaCO_3$)	170.81	57.57	255.39	106.51
Sulfate (mg/L)	58.45	7.19	1470.61	118.38
Sulfide (mg/L)	20.21	6.21	38.79	20.59
Chloride (mg/L)	25.18	1.70	435.00	9.77

Throughout the study, effluent pH in both sets of columns remained significantly higher than influent pH. It also generally remained in the 5-8 range considered optimal for SRB productivity and metal removal as sulfides (Postgate 1984, Elliot et al. 1998, Willow and Cohen 2003, Tsukamoto et al. 2004), with two exceptions. In weeks 44 and 50, average effluent pH in the HI columns were slightly above 8 at 8.18 and 8.06, respectively. pH in HI effluent was slightly, but significantly, higher than pH in LI effluent throughout the majority of the study, with means of 7.62 and 7.37, respectively. Both sets of columns exhibited significant upward trends in pH, with pH increasing from approximately 10 weeks until the end of the study.

Conductivity and alkalinity increased sharply in both sets of columns at the beginning of the study due to flushing of dissolved organic matter and inorganic base cations and anions contributed by amendments (chicken litter, lime, gypsum, etc.) in the spent mushroom compost, as shown in Figures 1.2 and 1.3 (Dvorak et al. 1992, Guo et al. 2001, Song et al. 2012). These parameters decreased quickly as the excess amendments and dissolved organic matter were flushed out of the substrate, with effluent conductivity returning to levels similar to the influent concentrations in both sets of columns within 12 weeks. Although alkalinity from both sets of columns decreased throughout the study, concentrations remained significantly higher than influent alkalinity.

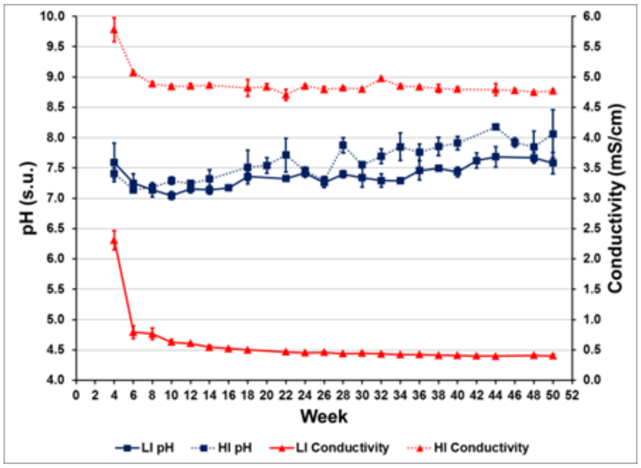


Figure 1.2 Averaged effluent pH and conductivity data for LI and HI columns ± one standard deviation

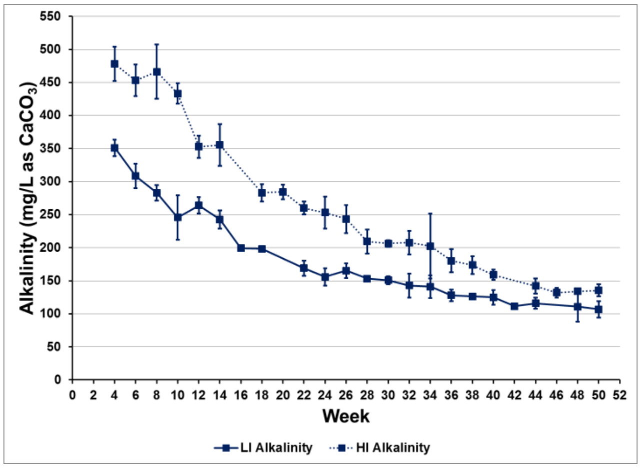


Figure 1.3 Averaged effluent alkalinity concentrations in LI and HI columns ± one standard deviation

Dissolution of $CaCO_3$ (in the form of sugar beet lime, a by-product of sugar beet processing) contained in the SMC was likely the largest contributor to the elevated effluent alkalinity in the beginning of the study, as evidenced by the concomitant increase in dissolved Ca concentrations in both the LI and HI columns shown in Figure 1.4 (Gross et al. 1993). Dissolved Ca concentrations remained elevated throughout the duration of the study, indicating continued dissolution of $CaCO_3$, but BSR likely became a larger contributor to alkalinity over time. The differences in effluent and influent sulfate and dissolved Ca concentrations were used to calculate equivalent alkalinities (as $CaCO_3$) and compared to measured alkalinity concentrations (Dvorak et al. 1992, Watzlaf et al. 2004). In LI columns, the equivalent alkalinity from dissolved Ca was very near the measured alkalinity. However, from approximately eight weeks into the study until the end, the addition of alkalinity from sulfate reduction was required to account for the remainder of the measured alkalinity. These comparisons are illustrated in Figure 1.5.

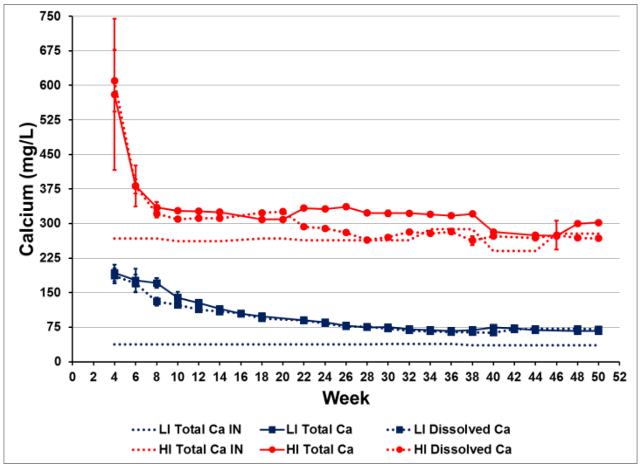


Figure 1.4 Influent and averaged Ca concentrations in LI and HI columns ± one standard deviation

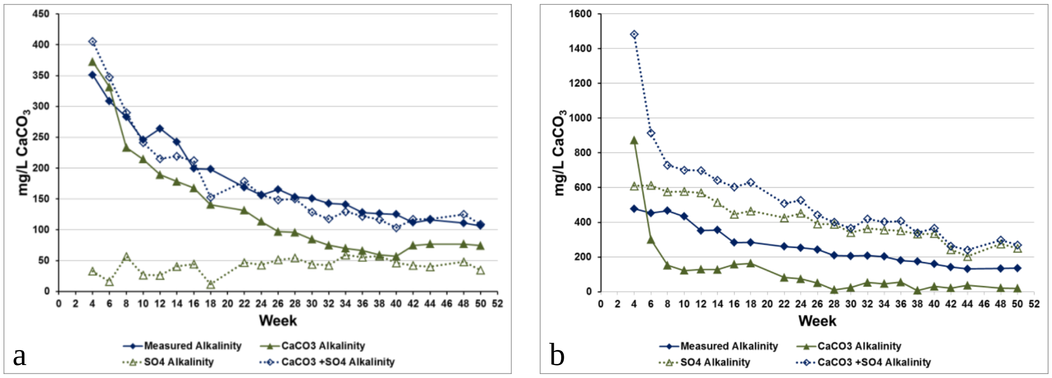


Figure 1.5 Alkalinity balance for LI (a) and HI (b) columns

Comparison of alkalinity contributions from $CaCO_3$ dissolution and sulfate reduction was not so straightforward in the HI columns. Dissolution of $CaCO_3$ made a much large contribution to alkalinity in the HI columns at the beginning of the study than it did in the LI columns. However, this contribution very quickly dropped near to values found in the LI columns, until approximately 20 weeks into the study, when $CaCO_3$ alkalinity in the HI columns fell below LI columns and remained there for the duration of the study. In addition, the calculated contribution of alkalinity from sulfate reduction was far greater in HI columns in LI columns, due to the much greater rate of sulfate removal.

Calculation of a sulfur balance in both sets of columns was performed in an attempt to understand the discrepancy in summed and measured alkalinities (Equation 1.1). Applying the assumption that all trace metals were removed as sulfides, an effluent mass of sulfur could be estimated and compared to the influent mass of sulfur.

$$\text{Influent}\left(\frac{\text{mmol S}}{\text{L}}\right) = \left[\text{Trace metal sulfide}\left(\frac{\text{mmol S}}{\text{L}}\right) + \text{Effluent sulfide}\left(\frac{\text{mmol S}}{\text{L}}\right) + \text{Effluent sulfate}\left(\frac{\text{mmol S}}{\text{L}}\right)\right] \qquad (1.1)$$

The results of the sulfur mass balance, shown in Table 1.5, indicated that total effluent masses exceeded influent masses in the LI columns throughout the study by an average of 0.37 moles, or 30%. This may be an indication that gypsum contained in the raw SMC dissolved, raising the porewater sulfate-S concentration and making it difficult to determine how much of the influent sulfur was actually removed. The calculations revealed the opposite relationship in the HI columns, with an average 3.05 mmol/L, or 17%, more sulfur in the influent than in the effluent. This indicates the possibility that gypsum may have been precipitating in the porewaters of these columns and effectively lowering the influent sulfate-S concentration and making accurate calculations of sulfate removal difficult. It is clear that by comparison of all sulfate and alkalinity data that sulfate reduction is not the only mechanism for removal of sulfate in the HI columns.

Table 1.5 Mass balance between mean influent and effluent sulfur concentrations (RPD = relative percent difference)

LI				**HI**			
S in mmol/L	**S out mmol/L**	**(in-out) mmol/L**	**RPD %**	**S in mmol/L**	**S out mmol/L**	**(in-out) mmol/L**	**RPD %**
1.08	1.44	-0.37	-30	19.59	16.92	3.05	17

Although gypsum may precipitate from mine waters high in Ca and sulfate, it was not assumed that concentrations would be great enough to create a supersaturated environment (Palmer et al. 2010). Speciation modeling in the geochemical modeling program PHREEQC (Parkhurst and Appelo 2013) indicated that both the LI and HI systems were approaching saturation with respect to gypsum, but both saturation indices were still negative (SI = -1.72 and -0.34, respectively). These analyses were conducted on the bulk effluent water quality and cannot account for the potential for supersaturated conditions in porewaters. Nonetheless, even if gypsum precipitation were occurring in the porewaters, it was not the sole cause of lower than anticipated effluent sulfate concentrations in the HI columns at the beginning of the study.

Other possible explanations for the greater sulfate removal in the HI columns are sorption of the $CaSO_4^0$ ion pair to the organic substrate, transformation of inorganic sulfate into organic sulfur, formation of elemental sulfur, or systemic error in measurements of influent sulfate concentrations (Bolan et al. 1991, Machemer et al. 1993, Sokolova and Alekseeva 2008). However, none of these potential mechanisms can be definitively accounted for or ruled out within the constraints of the data collected.

Sulfate and sulfide data were highly variable in both sets of columns, but the effect was not as apparent in the HI column effluents due to the much higher sulfate concentrations. These behaviors are illustrated in Figure 1.6. Although there was not a significant trend in effluent sulfate concentrations in the LI columns, there was a significant downward trend in effluent sulfide concentrations. This indicates that while sulfate was removed throughout the study, the dominant mechanism of removal may have shifted. In the HI columns, there was a significant upward trend in effluent sulfate concentrations coupled with a significant downward trend in sulfide concentrations. The mean sulfate removal rate in LI columns was 81 mmol/m^3/d, which is substantially lower than the 300 mmol/m^3/d that is expected under ideal conditions (URS 2003) and the 691 mmol/m^3/d found in the HI columns. Mean SRB populations throughout the study were between 10^2-10^3 organisms/mL in the LI columns and between 10^3-10^4 organisms/mL in the HI columns. Influent sulfate concentrations are known to impact SRB growth and sulfate reduction kinetics (Moosa et al. 2002), so a significant difference in LI and HI SRB populations and sulfate removal rates was anticipated. However, the calculated sulfate removal rate in the HI columns is more than likely artificially inflated due to reasons discussed previously.

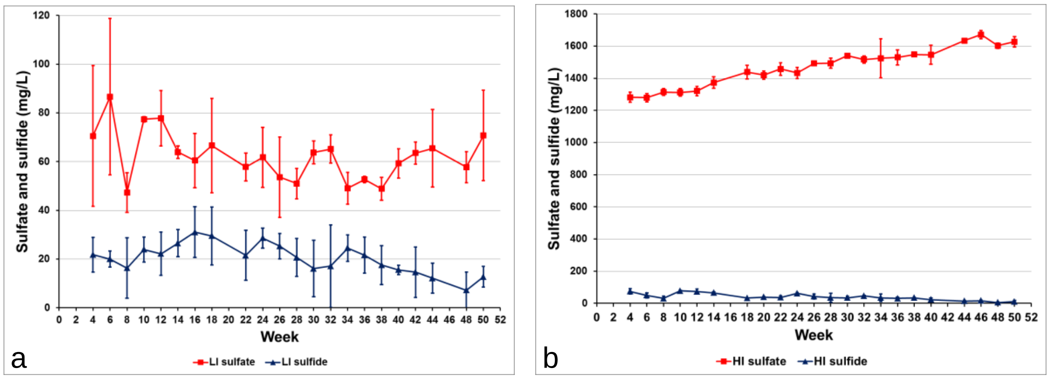


Figure 1.6 Averaged sulfate and sulfide concentrations in LI (a) and HI (b) columns ± one standard deviation

Total, inorganic, and organic carbon data were highly variable over the course of the study, as demonstrated by the calculated standard deviations in Table 1.6. However, this was due to the large impact of the initial flush of dissolved organic carbon, particularly in the first 12 weeks of operation. This influence is clearly illustrated in Figure 1.7. Statistically, total and dissolved carbon concentrations were not different. Surprisingly, despite the aforementioned potential for increased dissolution of $CaCO_3$ in the HI columns, there were no significant differences between any of the total or dissolved carbon fractions in LI and HI columns. Comparing inorganic carbon data to the alkalinity balance (Figure 1.5), 3-39% of inorganic carbon in the LI columns came from sulfate reduction, with the smallest contributions being made at the beginning of the study when the bulk of inorganic carbon came from $CaCO_3$ dissolution. Due to the uncertainties in the alkalinity balance for the HI columns, it was impossible to estimate the respective contributions of sulfate reduction and $CaCO_3$ dissolution to measured inorganic carbon.

Table 1.6 Mean effluent total, inorganic, and organic carbon data and standard deviations

	LI effluent (n = 22)				HI effluent (n = 22)			
	Total		**Dissolved**		**Total**		**Dissolved**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Total C (mg/L)	47.9	38.9	54.0	36.7	72.1	80.5	85.9	82.0
Inorganic C (mg/L)	34.6	19.1	39.5	18.2	43.0	42.2	56.6	43.0
Organic C(mg/L)	13.5	20.0	14.7	18.7	28.8	39.2	29.3	39.5

Despite the appearance that carbon concentrations may have been different in the LI and HI columns (Figure 1.7), there were no significant differences when considering the entirety of the data. However, dissolved inorganic carbon was significantly higher in the HI columns for approximately the first 12 weeks of the study. This is most likely due to the fact that elevated ionic strength increases the solubility product of $CaCO_3$, resulting in a greater amount of dissolution in the HI columns at the beginning of the study than in the LI columns (Stumm and Morgan 1993). Geochemical modeling indicated that approximately 30% of free Ca^{2+} ions in both the HI SMD and HI effluent were removed from solution as a result of ion pairing with sulfate, creating the $CaSO_4^0$ pair. This created a potential for enhanced dissolution of $CaCO_3$ in the HI columns (Drever 1997).

Effluent concentrations of calcium were significantly greater than influent concentrations in both sets of columns, even when removing the influence of the initial flush on effluent calcium concentrations evident in Figure 1.4. However, total and dissolved effluent calcium concentrations exhibited significant downward trends in LI and HI columns and nearly returned to influent concentrations in the HI columns. Calcium concentrations in LI columns remained elevated throughout the study, presumably due to continued dissolution of $CaCO_3$; whereas concentrations in the HI columns returned to near influent concentrations by the end of the study. This is likely due to a combination of quicker removal of $CaCO_3$ due to more rapid dissolution caused by elevated ionic strength and sorption of $CaSO_4^0$ ion pairs to the SMC.

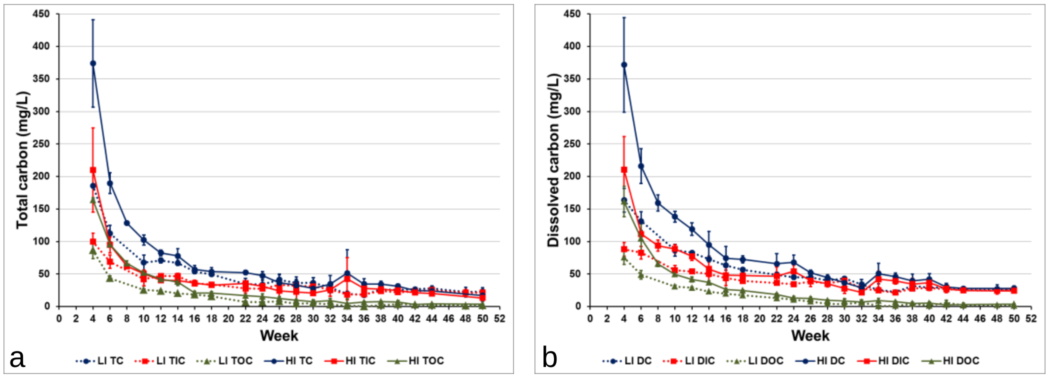


Figure 1.7 Averaged total (a) and dissolved (b) carbon (C), inorganic carbon (IC), and total organic carbon (OC) concentrations in LI and HI columns ± one standard deviation

Total and dissolved effluent sodium concentrations also exhibited a downward trend in the LI columns. In addition to calcium, a small amount of sodium appeared to leach out of the SMC for several weeks after the beginning of the study. In contrast, there were no significant trends in total or dissolved sodium concentrations in the HI columns.

With the exception of Mn, both sets of columns removed nearly all of the target trace metals (Table 1.7). Mean total and dissolved concentrations of Cd, Ni, Pb, and Zn all decreased significantly, from ~0.50 mg/L to below 0.10 mg/L, in both sets of columns. Manganese was not completely removed in either set of columns, with effluent concentrations in LI being significantly lower than effluent concentrations in HI.

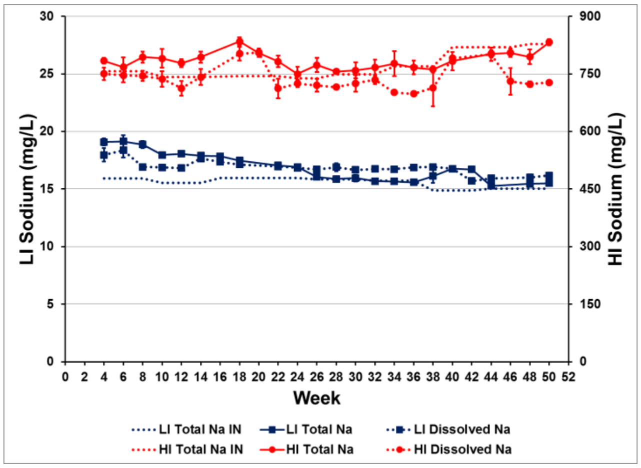


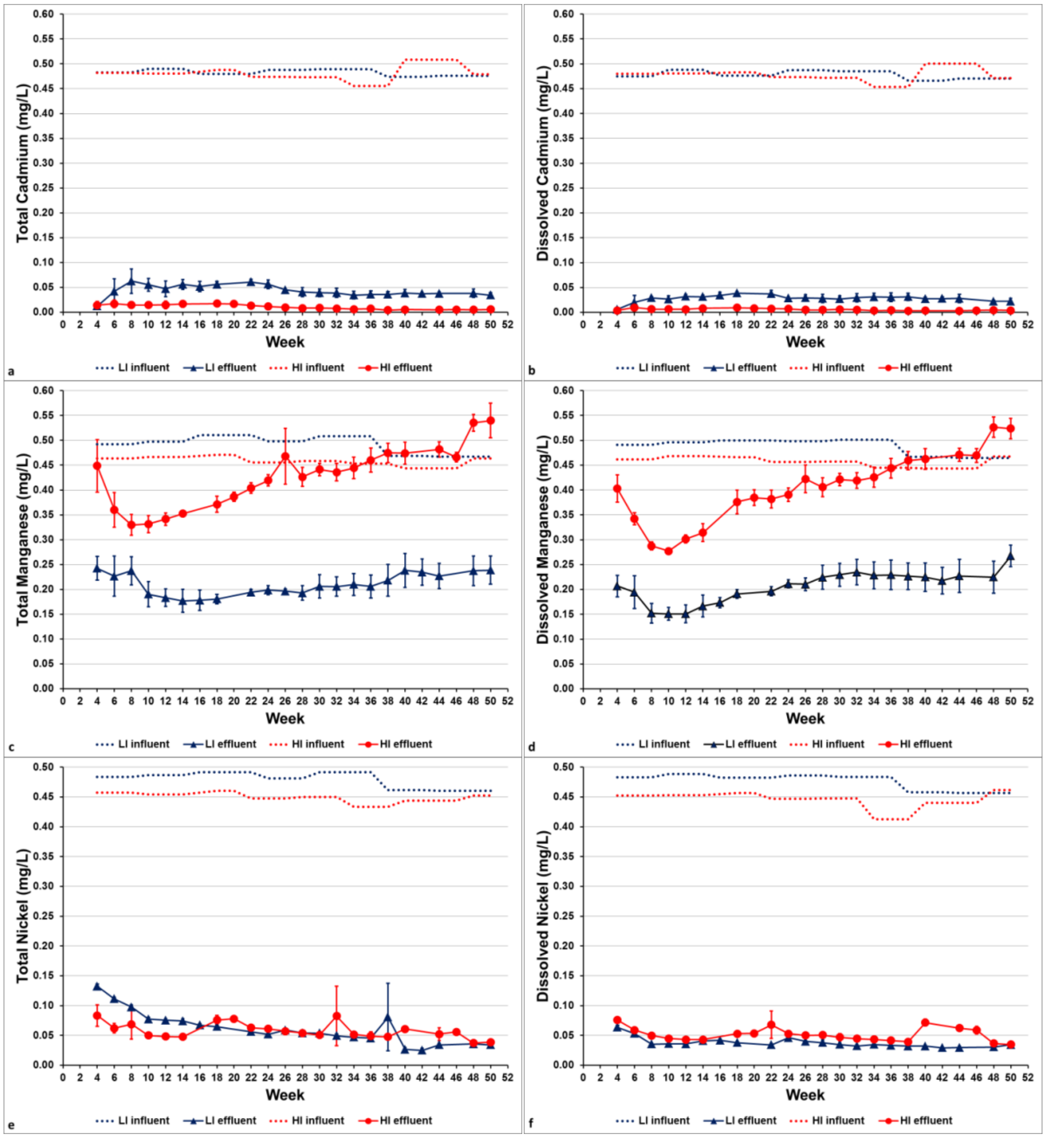
Figure 1.8 Influent and averaged sodium concentrations in LI and HI columns ± one standard deviation

Table 1.7 Mean effluent metals concentrations and standard deviations

	LI effluent (n = 22)				HI effluent (n = 22)			
	TOTAL		**DISSOLVED**		**TOTAL**		**DISSOLVED**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Cadmium (mg/L)	0.042	0.01	0.028	0.01	0.010	0.01	0.006	0.00
Manganese (mg/L)	0.211	0.02	0.206	0.03	0.427	0.06	0.405	0.07
Nickel (mg/L)	0.059	0.03	0.038	0.01	0.058	0.01	0.051	0.01
Lead (mg/L)	0.041	0.01	0.041	0.01	0.055	0.01	0.051	0.01
Zinc (mg/L)	0.067	0.02	0.042	0.01	0.035	0.03	0.017	0.00
Calcium (mg/L)	95.24	37.55	92.78	34.37	329.79	60.68	306.91	73.35
Sodium (mg/L)	16.72	1.25	16.86	0.613	784.51	22.43	739.25	32.48

It is well documented that, upon startup of any VFBR, a substantial portion of dissolved metals are removed via adsorption to the organic substrate (e.g. Machemer and Wildeman 1992, O’Sullivan et al. 2004, Stark et al. 2004, Gibert et al. 2005, Zagury et al. 2006, Neculita et al. 2008). This was the most likely explanation for the initial decrease in effluent Cd, Pb, and Zn concentrations in the LI columns. Total and dissolved Cd, Pb, and Zn concentrations in the LI effluent were all at their lowest in the first 2-4 weeks of the study, after which effluent concentrations of all three metals increased slightly (Figure 1.9). After sorption sites become saturated, precipitation as carbonates, hydroxides, and sulfides becomes the dominant removal mechanism. Past studies have demonstrated that the majority of Cd, Ni, Pb, and Zn are removed as sulfides, but large fractions may be removed as hydroxides and carbonates or through complexation with humic substances in the organic substrate (Dvorak et al. 1992, O'Sullivan et al. 2004, Chagué-Goff 2005, Neculita et al. 2008). Effluent sulfide, pH, alkalinity, and dissolved carbon concentrations were all sufficiently high enough in both sets of columns to support any of these removal mechanisms.

None of the trace metal effluent concentrations in the HI columns demonstrated the initial drop in concentration associated with adsorption seen in the LI columns. Whether this is due to an inhibition on adsorption or an enhancement of other removal mechanisms is unknown. Regardless, effluent concentrations of Cd and Zn were consistently significantly lower in HI columns than LI columns. Effluent Ni concentrations were significantly lower in LI columns, indicating elevated ionic strength had an inhibitory effect on removal. Effluent Ni concentrations in LI columns demonstrated a downward trend over the course of the study and effluent Cd, Ni, and Zn concentrations in HI columns demonstrated downward trends, indicating removal efficiency increased over the course of time. Conversely, Mn and Pb concentrations exhibited upward trends through the study, indicating a loss in removal efficiency.



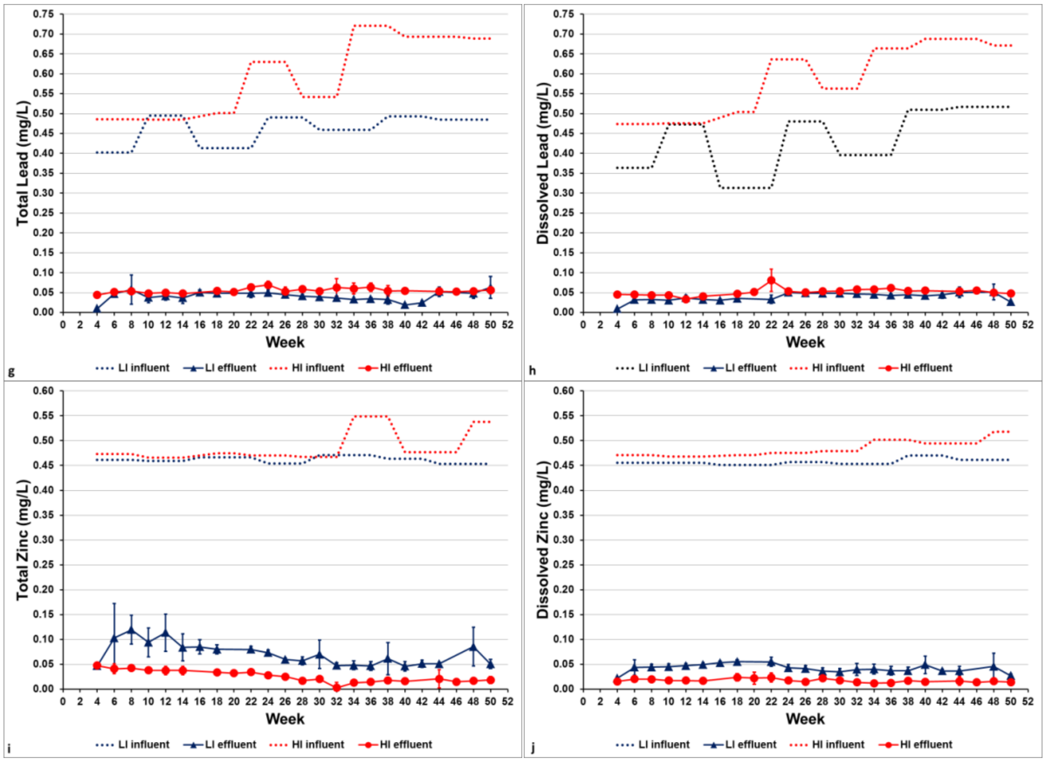


Figure 1.9 Influent and averaged metals concentrations in LI and HI columns; a) total Cd b) dissolved Cd c) total Mn d) dissolved Mn e) total Ni f) dissolved Ni g) total Pb h) dissolved Pb i) total Zn j) dissolved Zn ± one standard deviation

Effluent Pb concentrations were significantly lower in the LI columns than in the HI columns (Figure 1.9 g-h), though the influent concentrations were also significantly lower. Despite the differences between the columns, it is believed that the majority of Pb, as well as Cd, Ni, and Zn, were removed as sulfides and carbonates. Speciation modeling in PHREEQC indicated that effluents from both sets of columns were supersaturated with respect to Cd, Ni, Pb, and Zn sulfides and Cd and Pb carbonates, but were undersaturated with respect to hydroxides.

LI and HI effluents were not found to be supersaturated with respect to any Mn –sulfide, -carbonate, or -hydroxide minerals, indicating that the majority of removal was likely due to either adsorption to or complexation with the organic substrate.

Bless et al. (2008) found that adsorption of Mn onto organic substrates is an important part of removal, but that it is pH dependent and is often temporary. As pH increases, Mn may be replaced on the substrate by more readily adsorbed metals. Effluent Mn concentrations in LI columns were significantly lower than in HI columns (Figure 1.9 c-d). However, a similar trend was observed in both sets of columns, with a decrease in effluent concentrations for the first 10-12 weeks, after which time effluent concentrations began to increase. The increase in HI effluent Mn concentrations was much more pronounced than in LI columns, with the effluent concentration eventually exceeding the influent concentration. The increase in effluent concentrations strongly indicates adsorption (with subsequent desorption) as the removal mechanism, rather than complexation with organic matter. Whether this behavior was due directly to the elevated ionic strength or the slightly increased pH caused by the elevated ionic strength is unknown.

The relationships between water quality parameters and dissolved constituents of the effluents of LI and HI columns were evaluated using a Spearman’s rank correlation coefficient table. Significant positive and negative correlations between constituents are indicated in Table 1.8. Positive correlations between conductivity and sulfate, alkalinity, inorganic and organic carbon, calcium, and sodium were expected simply due to the definition of conductivity, as well as the large initial flush of dissolved organic matter and base cations and anions. Positive correlations between indicators of ionic strength (conductivity, Ca, Na, and sulfate) and Mn and Ni, however, reinforce the possibility that ionic strength impacts removal of trace metals in VFBR. These correlations provide more insight to the significant difference between LI and HI effluent concentrations, particularly with regard to Ni.

Table 1.8 Spearman’s rank correlation coefficient table for combined LI and HI effluent data (Bold values are significant.)

	Conductivity	**pH**	**DO**	**ORP**	**Sulfate**	**Alkalinity**	**Sulfide**	**DIC**	**DOC**	**Calcium**	**Sodium**	**Cadmium**	**Manganese**	**Nickel**	**Lead**
pH	0.07														
DO	0.07	**-0.56**													
ORP	**-0.64**	0.13	0.09												
Sulfate	**0.69**	**0.61**	-0.28	**-0.34**											
Alkalinity	**0.74**	**-0.44**	**0.52**	**-0.57**	0.18										
Sulfide	**0.75**	-0.24	0.13	**-0.76**	0.23	**0.77**									
DIC	**0.54**	**-0.49**	**0.48**	**-0.37**	0.03	**0.89**	**0.61**								
DOC	**0.62**	**-0.51**	**0.67**	**-0.47**	0.11	**0.93**	**0.70**	**0.86**							
Calcium	**0.94**	0.08	0.12	**-0.66**	**0.69**	**0.76**	**0.70**	**0.59**	**0.67**						
Sodium	**0.87**	0.22	0.03	**-0.54**	**0.73**	**0.59**	**0.54**	**0.43**	**0.47**	**0.88**					
Cadmium	**-0.68**	**-0.62**	**0.32**	**0.32**	**-0.86**	-0.20	-0.24	-0.07	-0.07	**-0.65**	**-0.67**				
Manganese	**0.58**	**0.69**	**-0.53**	**-0.38**	**0.85**	-0.04	0.19	-0.19	-0.18	**0.55**	**0.65**	**-0.86**			
Nickel	**0.74**	0.13	0.17	**-0.49**	**0.56**	**0.67**	**0.61**	**0.55**	**0.55**	**0.78**	**0.78**	**-0.57**	**0.42**		
Lead	0.30	**0.74**	**-0.52**	-0.22	**0.62**	-0.12	0.13	**-0.32**	-0.29	0.30	**0.31**	**-0.63**	**0.72**	**0.38**	
Zinc	**-0.70**	**-0.57**	**0.41**	**0.41**	**-0.80**	-0.18	**-0.32**	-0.01	-0.03	**-0.67**	**-0.66**	**0.90**	**-0.90**	**-0.50**	**-0.64**

Upon opening of the columns at the end of the study, it was noticed that the substrate had settled approximately 5 cm (2 in) and was covered by approximately 4 cm (1.5 in) of influent water. This led to an adjustment to the treatment volume used to calculate removal rates from 19.3 L to 16.7 L (0.0167 m^3). The treatment volume was considered equivalent to the volume of the submerged substrate. This value was used to calculate the volume adjusted removal rates listed in Table 1.9.

There were significant differences between all of the calculated removal rates in LI and HI columns. LI columns had significantly higher removal rates of Mn and Ni, which mirrored the significantly lower effluent concentrations found in those columns. Conversely, HI columns had significantly higher removal rates of Cd, Pb, and Zn.

Table 1.9 Average removal rates of total and dissolved Cd, Mn, Ni, Pb, and Zn in LI and HI columns

		Removal Rate (mg/m^3/d)			
		LI		HI	
		Mean	**SD**	**Mean**	**SD**
Cadmium	Total	85.4	2.1	87.4	0.9
	Dissolved	87.3	1.3	87.7	0.4
Manganese	Total	54.4	4.8	6.1	11.4
	Dissolved	54.7	6.0	9.9	12.9
Nickel	Total	81.7	5.1	73.0	2.4
	Dissolved	85.2	1.6	73.6	2.1
Lead	Total	82.2	2.6	100.3	1.1
	Dissolved	77.1	2.3	99.2	1.7
Zinc	Total	76.5	4.0	84.6	5.2
	Dissolved	80.7	1.6	87.0	0.6

Conclusions

Elevated ionic strength appeared to have significant impacts on some inorganic constituents of the study, namely pH, alkalinity, and trace metal removal. pH and alkalinity impacts were most likely due to increased solubility of $CaCO_3$ contained in the SMC, but may have also been affected by the greater sulfate concentrations in the HI columns. There were no evident effects on the two organic constituents evaluated, carbon concentrations and SRB populations. It was not anticipated that elevated ionic strength (TDS/conductivity) would impact the five selected trace metals differently. However, it was found that elevated ionic strength increased the rate of removal of Cd and Zn, and possibly Pb. Elevated ionic strength decreased the rate of removal of Ni and greatly decreased the rate of removal of Mn as well as caused the eventual release of Mn from the substrate. Manganese concentrations by far demonstrated the most difference between LI and HI columns. Mechanisms for removal were not determined in this portion of the study, but a subsequent study on the spent substrates will elucidate more clearly the impact of elevated ionic strength on products of trace metal removal in VFBR.

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Chapter Two: Evaluating the impact of Na-SO_4 dominated ionic strength on trace metal removal products in bench-scale vertical flow bioreactors using sequential extractions, acid-volatile sulfide analyses, and mineralogical methods

This chapter has been formatted and submitted to *Applied Geochemistry.*

Abstract

Vertical flow bioreactors (VFBR) are often used as a component of passive treatment systems (PTS) to treat mine drainage. These mine drainages generally contain elevated concentrations of Fe, sulfate, and trace metals. One of the primary purposes of VFBR is to remove trace metals from mine drainage and retain them in the organic substrate. Elevated ionic strength may impact the performance of VFBR and affect their ability to remove trace metals. A paired-comparison study was performed in which two sets of columns were constructed, filled with an organic substrate, and fed synthetic mine drainage of differing ionic strengths, dominated by sodium and sulfate, for one year. Substrate samples were collected and analyzed for total Cd, Mn, Ni, Pb, and Zn and subjected to a sequential extraction procedure (SEP) and acid-volatile sulfide/simultaneously extracted metals (AVS/SEM) analyses. There were no differences in the total metals concentrations between the two sets of columns. There were, however, differences between how some of the metals were retained within the substrates. Elevated ionic strength resulted in less Cd being retained as an insoluble sulfide, while the opposite was true for Pb. In addition, increased ionic strength caused a decrease in the amount of Pb retained in the labile organic fraction. The SEM/AVS ratio indicated that a large portion of Cd, Mn, Ni, Pb, Zn was likely in soluble and

bioavailable forms. When all of the removal products are considered, Ni retention within the substrate was the most stable and Mn was the least stable of the trace metals examined.

Introduction

Vertical flow bioreactors (VFBR), also known as biochemical reactors, are an emerging technology used in the treatment of hard rock mine drainages. Although they were originally intended as a process to promote alkalinity generation via bacterial sulfate reduction (BSR) and dissolution of limestone mixed with or underlying an organic substrate (Kepler and McCleary, 1994), they have also been used to remove trace metals often associated with mine drainage. Trace metals may be removed from mine drainage through a variety of mechanisms, such as adsorption to organic matter, carbonates, or other precipitates, complexation with organic matter, and precipitation as sulfides, hydroxides, and carbonates (Dvorak et al. 1992, Machemer and Wildeman 1992, Webb et al. 1998, Neculita et al. 2008b). Large concentrations of sulfate may also be removed from mine drainage in these systems, depending upon hydraulic retention time and type of substrate used (Dvorak et al. 1992, Cocos et al. 2002, Willow and Cohen 2003, Neculita et al. 2007, Pinto et al. 2015).

Precipitation of trace metals as sulfides is considered the most desirable removal mechanism in VFBR due to the stability and decreased bioavailability of the sulfides under reducing conditions (Machemer and Wildeman 1992, Wildeman and Updegraff 1998, Song et al. 2001, Bless et al. 2008). This process is mediated through BSR; using the decomposed organic substrate (CH_2O) as a carbon source and electron donor and sulfate in the mine water as the terminal electron acceptor, sulfate reducing bacteria (SRB) reduce sulfur from S^{6+} to S^{2-}, producing soluble sulfides (H_2S, HS^-, and S^{2-}). The reaction, including the production of bicarbonate through BSR, is shown in Equation 2.1.

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S \tag{2.1}$$

The soluble sulfide will then react with divalent cationic metals and bicarbonate formed in Equation 2.1 to form metal sulfide precipitates, show in Equation 2.2.

$$H_2S + M^{2+} + HCO_3^- \rightarrow MS\downarrow + 2H_2O + 2CO_2 \tag{2.2}$$

The oxidation and dissolution of pyrite and other sulfide minerals, as well as secondary reactions and ion exchange processes that occur in flooded mines contribute elevated cations (Fe^{2+}, Ca^{2+}, Mg^{2+}, Na^+) and sulfate concentrations to mine drainage (Capo et al. 2001, Younger et al. 2002, Watzlaf et al. 2004). The bulk of the inorganic portion of total dissolved solids (TDS) in mine drainages is typically made up of base cations (Na^+, Ca^{2+}, Mg^{2+}), sulfate, and bicarbonate alkalinity (Skousen et al. 2000, Palmer et al. 2010, Timpano et al. 2010). Along with conductivity and ionic strength, TDS is known as a sum parameter that represents the sum total of all anions and cations in a solution (Hem 1985, Cravotta 2008, Cravotta and Brady 2015). The USEPA has suggested rules for the regulation of TDS concentrations in natural waters, but treatment to remove TDS to proposed limits is troublesome (USEPA 2011). Pinto et al. (2015) found that TDS removal through typical mine drainage treatment methods can be inconsistent. In addition to issues with TDS removal, it is also possible that elevated TDS concentrations could affect some methods of treatment or trace metal removal (Hem 1985, Langmuir 1997, Cravotta 2008).

Elevated ionic strength, conductivity, and TDS may positively or negatively affect trace metal removal in VFBR. Precipitation of divalent trace metals as carbonates is a potential mechanism of removal due to an increase in bicarbonate concentration and pH as a result of BSR (Dvorak et al. 1992, Sobolewski 1996). However, it has been observed that an increase in ionic strength may increase the solubility of metal-carbonate precipitates, causing trace metals to remain in solution or be removed by different mechanisms (Plassard et al. 2000, Sun et al. 2009). Although the solubility of sulfide minerals theoretically increases as ionic strength increases (Lewis 2010), it may be possible for the opposite to occur for at least some sulfide minerals (Shpiner et al. 2009). Shpiner et al. (2009) theorized that an increase in ionic strength could cause a decrease in double-layer repulsive forces, allowing particles to get close enough for van der Waals forces to take over and resulting in lower solubility. More fundamentally, the possibility for precipitation of trace metals as sulfides may be limited by potential ionic strength impacts on hydrogen sulfide solubility (Duan et al. 2007).

The final products of removal can have a profound impact on the stability and bioavailability of trace metals, making it highly desirable to confirm their identity. Sequential extraction procedures are operationally defined methods used for the speciation of trace metals retained in substrates. A sequence of selective chemical extraction fluids is used to release species associated with specific solid phases, with each step being more vigorous than the previous one (Filgueiras et al. 2002, Gleyzes et al. 2002). In general, trace metals can be placed into five categories: i) adsorptive and exchangeable, ii) bound to carbonates, iii) bound to Fe/Mn oxides, iv) bound to organic matter and sulfides, and v) bound to primary and secondary minerals (Tessier et al. 1979, Filgueiras et al. 2002, Gleyzes et al. 2002, Jong and Parry 2004).

Another important tool in evaluating the presence of sulfide in spent substrates is the determination of acid-volatile sulfide/simultaneously extracted metals (AVS/SEM). Substrates are treated with hydrochloric acid and the liberated AVS and SEM are collected and measured (Brouwer and Murphy 1994, Leonard et al. 1996). Although results may be complicated by the fact that other fractions present in substrates may also be extracted and some sulfides (particularly CuS and NiS) do not dissolve well in hydrochloric acid, AVS/SEM analyses can serve as an important complement to other solid-phase analyses (Jong and Parry 2004).

Solid-phase mineralogical analyses of VFBR substrates can be troublesome due to poor crystallinity of precipitates and low concentrations of removal products. However, some long-term studies have had success using scanning electron microscopy to identify precipitates in VFBR substrates (Machemer et al. 1993, Gibert et al. 2005, Neculita et al. 2008b). Mineralogical analyses may be used to confirm results found in chemical speciation methods, allowing positive identification of trace metal removal products.

Methods and Materials

Experimental design

A paired-comparison column study was designed to assess the effect of ionic strength on trace metal removal products in VFBR. A hydraulic retention time of 72 hours, a target flow rate of approximately 3 L/day, and an assumed porosity of 0.5 were used to determine the dimensions of the columns. Two sets of four continuously fed, downward-flow bench-scale VFBR were constructed using opaque, 25-cm diameter PVC pipe with a length of 38-cm. The final capacity of the columns was 19.3 L and each was filled with a 2:1 mixture (by volume) of spent mushroom compost (SMC) and inert river rock. This mixture was placed on top of a 2.5 cm layer of inert pea gravel that was placed at the bottom of the columns to prevent substrate from entering and collecting in the effluent tubing. The SMC, from J-M Farms, Inc. in Miami, OK, consisted of a base of wheat straw amended with chicken litter, cottonseed meal, soybean meal, peat moss, sugar beet lime, and gypsum.

The two sets of columns were differentiated by the ionic strength of the simulated mine drainage (SMD) fed into them. One set received SMD of “low” ionic strength at approximately 10^{-3} M (LI) and the other set received “high” ionic strength SMD at approximately 10^{-1} M (HI). All SMD was designed to simulate common field conditions after Fe has been removed via oxidation, hydrolysis, and precipitation from a net-alkaline mine drainage: net-neutral acidity/alkalinity, circum-neutral pH, elevated sulfate and trace metals concentrations. Each SMD contained 0.5 mg/L each of Cd, Mn, Ni, Pb, and Zn. LI SMD contained smaller base cation and anion concentrations, with 10 mg/L Ca, 25 mg/L Na, 15 mg/L Cl, and 125 mg/L sulfate; whereas HI SMD contained greater base cation and anion concentrations, with 250 mg/L Ca, 1000 mg/L Na, 440 mg/L Cl and 2100 mg/L sulfate.

In addition to three columns each receiving LI and HI SMD, an additional “kill” column was added to each set. Ammonium molybdate (0.2 mM) was added to a fourth “low” ionic strength column (LK) and a fourth “high” ionic strength column (HK), with the intention to inhibit SRB growth. The objective of these two additional columns was to determine if trace metals would continue to be removed in the absence of BSR and/or if the proportions of trace metal removal products would remain the same.

LI and HI SMD were prepared in 50-L carboys fresh approximately every five days, while LK and HK were prepared in 20-L carboys and were prepared fresh approximately every 8 days. SMD were fed into each of their respective columns with peristaltic pumps at a target flow rate of 3.1 L/d (130 mL/hour) and allowed to flow naturally via gravity through the remainder of the system.

Sampling and analysis

Following an adjustment period of four weeks to allow for anoxic, reducing conditions to be established, effluent samples were collected from each column approximately every two weeks. LI and HI columns were allowed to run for 52 weeks (approximately equivalent to 100 pore volumes) and LK and HK columns were allowed to run for 34 weeks. Temperature, pH, dissolved oxygen, conductivity, oxidation-reduction potential, and total alkalinity were measured immediately upon sample collection. Sulfide and sulfate samples were analyzed within 24 and 48 hours, respectively. Water quality parameters were measured using a benchtop Accumet multi-meter, total alkalinity and sulfate were analyzed following Hach methods 8203 and 8051, respectively, and sulfide was analyzed following APHA method 4500 D.

Total and dissolved metals samples were collected in 60-mL HDPE bottles and acidified with trace metal grade nitric acid to pH < 2. Dissolved metals samples were filtered with 0.45-µm nylon syringe filters prior to acidification. All metals samples were microwave digested with nitric acid according to EPA method 3015 and analyzed for Ca, Cd, Mn, Na, Ni, Pb, and Zn with a Varian Vista-PRO ICP-OES according to EPA method 6010. Total and dissolved carbon samples were collected in 40-mL amber glass bottles and stored at < 4°C until analysis. Carbon samples were analyzed with an Analytik Jena N/C 2100 carbon analyzer according to EPA method 9060. Additional samples were collected approximately quarterly to determine SRB populations. Bacteria culture bottles containing the American Petroleum Institute RP-38 sulfate reducer medium were obtained from VK Enterprises (Edmond, OK), inoculated, and incubated at room temperature to determine approximate populations.

While the column study was being conducted, a separate sample of SMC was saturated with water and allowed to soak 30 days to ensure complete saturation. Bulk density and particle density of the SMC were evaluated at the end of this time.

Bulk density was determined according to Methods of Soil Analysis (Blake and Hartge 1986) and particle density was determined following Weindorf and Wittie (2003). Porosity was calculated using these two parameters according to Danielson and Sutherland (1986).

Before the study began, samples of the original SMC were collected for later analyses. By the end of the study, the substrate had settled approximately 5 cm in each column, resulting in an average substrate depth of 30 cm. Substrate samples were collected from 0-10 cm (top), 10-20 cm (middle), and 20-30 cm (bottom). Samples were collected by hand before water was completely drained from the columns, to prevent oxygen infiltration into samples. Each substrate sample was immediately placed in a vacuum zipper bag, which was quickly sealed and evacuated with a FreshSaver® Handheld Vacuum System. These bags were placed in another zipper bag, sealed, and vacuumed. All substrate samples were stored at < 4°C until analyses were performed.

Moisture content analyses were conducted in triplicate on each substrate sample. Samples were dried for 24 hours at 60°C. After drying and weighing, the moisture content samples were lightly ground to < 2 mm and retained for total metals, carbon, and mineralogical analyses. Subsamples of the dried substrate samples were pretreated with nitric and hydrochloric acids for 12 hours under a fume hood before microwave digestion according to EPA method 3051. All digested substrate samples were analyzed for Ca, Cd, Mn, Na, Ni, Pb, and Zn with a Varian Vista-PRO ICP-OES according to EPA method 6010. Subsamples of the dried substrate samples were also analyzed for total, inorganic, and organic carbon content using an Analytik Jena N/C 2100 carbon analyzer according to EPA method 9060.

Acid-volatile sulfide/simultaneously extracted metals (AVS/SEM) tests were performed in triplicate on each substrate sample. AVS/SEM analyses were conducted following Leonard et al. (1996), using a modified diffusion method developed by Brouwer and Murphy (1994). The procedure was carried out in 125-mL glass bottles with 30-mL vials glued to the side, approximately 2 cm from the bottom. A sulfide antioxidant buffer (SAOB) containing 2 M sodium hydroxide, 0.2 M disodium ethylenediaminetetraacetic acid, and 0.2 M ascorbic acid was prepared daily using nitrogen-sparged ultrapure water and used to collect hydrogen sulfide gas produced during the procedure. For each analysis, 10 mL SAOB was placed in the vial. Substrate samples (1-2 g dry weight) and 10 mL 1 N hydrochloric acid were placed in the bottles, which were capped immediately and lightly agitated on a table shaker for 1 hour. At the end of 1 hour, SAOB samples (AVS) were analyzed for sulfide following APHA method 4500 D and hydrochloric acid extracts (SEM) were filtered using 0.45-μm polypropylene syringe filters and collected in 60-mL HDPE bottles. Metals samples were analyzed for Cd, Mn, Ni, Pb, and Zn via ICP-OES.

A sequential extraction procedure (SEP) consisting of six operationally defined fractions was conducted in triplicate on each substrate sample. The SEP included fractions from Tessier et al. (1979) as well an extra labile organic fraction from Hall et al. (1996). The labile organic fraction was added to target the large amount of humic and fulvic acids that are contained in the substrate and expected to contribute significantly to trace metal removal. With the addition of this step, the organic contribution to the fifth step would be greatly, if not completely, diminished. The order of the extractions was intended to target increasingly stable constituents as the SEP progressed. Due to the elevated concentrations of carbonates and labile organic matter expected to be found in the substrates, steps 2 and 3 were performed twice and the extracts combined into one sample. The fractions determined were:

1. Exchangeable – This fraction consists of metals that may be released through ion-exchange processes and those that are weakly adsorbed to the substrate surface (Tessier et al. 1979, Filgueiras et al. 2002, Gleyzes et al. 2002). The procedure calls for the addition of 8-mL of 1 M $MgCl_2$ adjusted to pH 7 and agitation of the sample for1 hour.
2. Bound to carbonate – This fraction consists of metals which are precipitated or co-precipitated with carbonate, as well as metals adsorbed to carbonate surfaces (Tessier et al. 1979, Filgueiras et al. 2002, Gleyzes et al. 2002). The procedure calls for the addition of 20-mL of 1 M NaOAc adjusted to pH 5 with acetic acid to residue from (1) and agitation of the sample for 1 hour.
3. Bound to labile organic matter – This fraction consists of metals which are bound up in humic and fulvic acids through complexation (Papp et al. 1991, Krishnamurti et al. 1995, Hall et al. 1996). The procedure calls for the

addition of 30-mL of 0.1 M $Na_4P_2O_7 \cdot 10H_2O$ adjusted to pH 10 to residue from (2) and agitation of the sample for 1 hour.

4. Bound to Fe/Mn oxides – This fraction consists of Fe and Mn oxides and any metals that may be adsorbed to them. Given that anoxic substrates analyzed in this study should contain little to no oxide content, the agitation time of 6 hours in Tessier et al. (1979) was shortened to 1 hour after Chester et al. (1988) and Arunachalam et al. (1996). The procedure calls for the addition of 20-mL of 0.04 M $NH_2OH \cdot HCl$ in 25% (v/v) acetic acid to residue from (3) and agitation of the sample for 1 hour.
5. Bound to refractory organic matter/sulfides – This fraction consists of metals that are bound to sulfides and decay-resistant organic matter with low solubility (Tessier et al. 1979, Hall et al. 1996). The procedure calls for the addition of 3-mL of 0.02 M HNO_3 and 5-mL 30% H_2O_2 adjusted to pH 2 with HNO_3 to residue from (4), which is then heated to 85±2°C for 2 hours with occasional agitation After this time, an additional 3-mL 30% H_2O_2 is added and heated to 85±2°C for 3more hours with occasional agitation. Samples are then cooled, and 5-mL of 3.2 M NH_4OAc in 20% (v/v) HNO_3 and 4-mL sparged ultrapure water are added and the sample is agitated for 30 min.
6. Residual fraction – This fraction consists of metals that are bound to primary and secondary minerals, particularly silicates, which are typically released in the environment as a result of weathering (Tessier et al. 1979, Filgueiras et al. 2002). Given the highly degradable nature of the substrates

as a whole, the more hazardous HF-$HClO_4$ digestion was substituted with a less rigorous acid. The residue from (5) was microwave digested with 10-mL concentrated HNO_3.

All extractions were carried out in a nitrogen-filled anaerobic chamber to prevent oxidation of the substrate and alteration of fractions. Samples were not dried and, as a result, the exchangeable fraction was expected to also contain any trace metals contained in the pore waters. Approximately 1 g (dry weight) subsamples were subjected to the SEP. All extraction fluids were sparged with nitrogen prior to extraction in order to prevent oxidation of the anaerobic substrates.

Following each fraction, samples were centrifuged at 10000 rpm for 15 minutes and the supernatant collected in 60-mL HDPE bottles. Samples were then rinsed with 10-mL sparged ultrapure water, centrifuged again for 15 minutes, and the rinse collected and added to the supernatant from that fraction. Samples were then subjected to the next extraction step. All SEP samples were filtered with 0.45-µm polypropylene syringe filters and analyzed for Cd, Mn, Ni, Pb, and Zn via ICP-OES. Duplicate blanks and matrix spikes were included in each ICP-OES analysis to assess potential matrix effects and method accuracy.

Dried samples were prepared for mineralogical analyses by mounting on Al stubs with carbon tape and sputter-coating with Au-Pd alloy. Analysis was conducted with a Zeiss NEON 40 EsB field emission scanning electron microscope fitted with an INCA Energy 250 energy dispersive x-ray microanalysis system (SEM-EDS) and an Oxford backscatter detector.

Statistics

The statistical software package Minitab version 17.2.1was used to compute all statistics (Minitab 2010). A significance level of 0.05 was applied. All data sets were checked for normality using the Anderson-Darling statistic. The Mann-Whitney test (also known as the Wilcoxen rank sum test) was used to determine significant difference between two sets of data. Differences between more than two sets of data were found using the Kruskal-Wallis test.

Results

Quality control

The precision and accuracy of all metals analyses via ICP-OES were assessed by analyzing at least one duplicate sample and one spike for each batch of analyses, for a total of 22 duplicates and 22 spikes. Recovery of Cd, Mn, Ni, Pb, and Zn ranged from 95-109% in duplicates and 93-105% in spikes, when the concentrations were at least ten times greater than the detection limits. Detections limits for each metal in each procedure are listed in Table 2.1.

Table 2.1 ICP-OES detection limits for aqueous and extracted solid samples

	Cd	**Mn**	**Ni**	**Pb**	**Zn**
Aqueous (µg/L)	0.64	0.21	4.0	19	1.3
Total metals (µg/kg)	31	10	19	94	6.1
AVS/SEM (µg/kg)	6.4	2.1	40	190	13
SEP (µg/kg)[a]	6.4-45	2.1-15	40-280	190-1400	13-89

[a] Limits vary depending upon volume of extraction fluid in each step of the SEP; steps with a smaller volume have lower DL

Influent water quality

Over the course of the study, a total of eight sets of samples from LI and HI SMD were collected. In addition, four sets of samples from LK and HK SMD were collected. All SMD exhibited circumneutral pH (~6.0) and contained less than 15 mg/L alkalinity. Conductivity was significantly higher in HI SMD as was expected due to the abundance of anions and cations, but it was also significantly higher in LK and HK SMD. This was presumably due to the addition of 0.2 mmol ammonium molybdate. In some cases, metals concentrations differed from the target concentrations (Table 2.2). This was either due to contamination in a salt used to produce the SMD (Pb in HI) or incomplete dissolution or precipitation of a salt (Cd and Pb in LK and HK).

Table 2.2 Mean (± one standard deviation) influent water quality and dissolved metals concentrations for all sets of columns

	LI (n = 8)		**HI (n = 8)**		**LK (n = 4)**		**HK (n = 4)**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Temperature (°C)	21	0.54	21	0.64	21	0.70	21	0.66
Conductivity (mS/cm)	0.32	0.01	4.9	0.14	1.44	0.16	5.4	0.08
pH (s.u.)	6.3	0.11	6.0	0.03	6.4	0.09	5.9	0.01
DO (mg/L)	8.1	0.45	8.3	0.20	8.1	0.48	8.3	0.14
ORP (mV)	214	15	186	39	218	16	199	24
Alkalinity (mg/L as $CaCO_3$)	5.7	3.0	3.2	0.32	13	0.35	4.25	021
Sulfate (mg/L)	104	1.5	1830	54	105	1.4	1840	61
Cadmium (mg/L)	0.48	0.01	0.48	0.01	0.34	0.03	0.32	0.10
Manganese (mg/L)	0.49	0.02	0.46	0.01	0.44	0.02	0.42	0.01
Nickel (mg/L)	0.48	0.01	0.45	0.02	0.51	0.01	0.40	0.01
Lead (mg/L)	0.44	0.08	0.58	0.09	0.36	0.06	0.21	0.01
Zinc (mg/L)	0.46	0.01	0.49	0.02	0.44	0.01	0.58	0.13
Calcium (mg/L)	39	1.4	260	13	35	1.6	230	16
Sodium (mg/L)	16	0.23	707	31	15	0.05	683	26

Effluent water quality

Effluent water quality data are summarized in Table 2.3. All columns very quickly developed the anaerobic and reducing conditions necessary for BSR, with DO < 1 mg/L and ORP < -100 mV. Effluent alkalinity and pH were elevated in all columns, with pH an order of magnitude greater in the LI and HI columns than in the LK and HK columns. With the exception of Mn, trace metals were nearly completely removed (< 0.1 mg/L) in the LI and HI columns. Removal efficiency of all five trace metals was more variable in the LK and HK columns, with mean effluent Mn concentrations (0.91 ± 0.45 and 1.2 ± 0.17 mg/L, respectively) exceeding the influent concentrations (0.44 and 0.42 mg/L, respectively) through nearly the entire study. Some sulfate was removed from LI, HI, and HK columns, but not from the LK column. Mean SRB populations were 10^2-10^3 organisms/mL in the LI effluent, 10^3-10^4 organisms/mL in the HI effluent, and undetectable in the LK and HK effluents. These data, coupled with the production of sulfide in the LI and HI columns, indicate that BSR was occurring in the LI and HI columns, but not in the LK and HK columns. The great degree of sulfate removal in the HK column without the concomitant production of sulfide indicates that another mechanism of removal may be occurring. Adsorption to organic matter, transformation of inorganic sulfate into organic sulfur, and/or formation of elemental sulfur may also be playing a large role in sulfate removal (Bolan et al. 1991, Machemer et al. 1993, Sokolova and Alekseeva 2008). Sulfate removal seen in HI columns was likely through a combination of these possibilities and BSR. Despite the addition of the

SRB inhibitor, Cd, Ni, Pb, and Zn were removed to some degree in the LK and HK columns, indicating the potential for removal products other than sulfides.

Table 2.3 Mean (± one standard deviation) effluent water quality and dissolved metals concentrations from all column sets

	LI (n = 22)		HI (n = 22)		LK (n = 11)		HK (n = 11)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Temperature (°C)	21	0.34	21	0.40	21	0.36	21	0.45
Conductivity (mS/cm)	0.57	0.40	4.9	0.22	1.7	0.29	5.6	0.25
pH (s.u.)	7.4	0.18	7.6	0.30	6.3	0.58	6.3	0.60
DO (mg/L)	0.21	0.34	0.04	0.09	0.38	0.85	0.46	0.78
ORP (mV)	-260	71	-314	34	-29	21	-27	30
Alkalinity (mg/L as $CaCO_3$)	180	71	260	110	530	150	520	200
Sulfate (mg/L)	59	7.2	1470	120	110	9.5	1410	48
Sulfide (mg/L)	20	6.1	39	21	-	-	-	-
Cadmium (mg/L)	0.03	0.01	0.01	0.00	0.01	0.00	0.03	0.04
Manganese (mg/L)	0.21	0.03	0.41	0.07	0.91	0.45	1.2	0.17
Nickel (mg/L)	0.04	0.01	0.05	0.01	0.12	0.02	0.09	0.03
Lead (mg/L)	0.04	0.01	0.05	0.01	0.23	0.04	0.21	0.01
Zinc (mg/L)	0.04	0.01	0.02	0.00	0.07	0.04	0.12	0.11
Calcium (mg/L)	93	34	310	73	220	53	410	91
Sodium (mg/L)	17	0.61	740	32	18	1.6	680	37

All columns performed relatively consistently throughout the duration of the study with respect to Cd, Ni, Pb, and Zn concentrations. Loading and removal rates and percent removal of trace metals, sulfate and Ca are shown in Table 2.4. Negative values indicate a release of the constituent. All columns released Ca due to dissolution of $CaCO_3$ in the SMC, but the effect was greater in the LK and HK columns. Overall, LI columns removed more trace metals, with the exception of Cd, and sulfate than the LK column. However, Figure 2.1 shows that Mn and Pb concentrations increased in LI over the course of the study and Cd, Mn, Ni, and Zn concentrations increased in the LK column, demonstrating decreased removal efficiency in both sets of columns. HI columns removed more of all five trace metals than the HK column, but sulfate removal was nearly the same. This enhances the likelihood that a large portion of the observed sulfate removal was due to adsorption to organic matter.

Table 2.4 Mean loading and removal rates of trace metals, calcium, and sulfate in all sets of columns; negative removal rates indicate a release of the constituent

	Loading Rate ($mg/m^3/d$)				**Removal Rate ($mg/m^3/d$)**				**% Removed**			
	LI	**HI**	**LK**	**HK**	**LI**	**HI**	**LK**	**HK**	**LI**	**HI**	**LK**	**HK**
Cd	93	89	64	60	87	91	62	55	94	98	97	91
Mn	95	86	81	78	54	10	-87	-140	57	11	-110	-180
Ni	93	84	94	74	85	78	72	58	92	89	77	78
Pb	85	110	66	40	78	100	22	-0.40	91	91	34	-1
Zn	89	91	81	110	81	91	68	87	91	96	84	80
Ca	7.5	48	6.6	190	-10.6	-9.8	-35	-34	-140	-19	-530	-78
Sulfate	20	340	19	1500	8.7	69	-0.56	78	43	20	-3	23

Physical characteristics

Given the inundated conditions of the substrates and the method of collection, moisture contents were used to calculate dry masses of the wet subsamples used in the AVS/SEM and SEP analyses. Median moisture content for all of the substrate samples was 87%. All values fell within a range of 78% and 91%, with an interquartile range of 85% and 89%. All trace metal concentrations are presented as per dry mass of substrate.

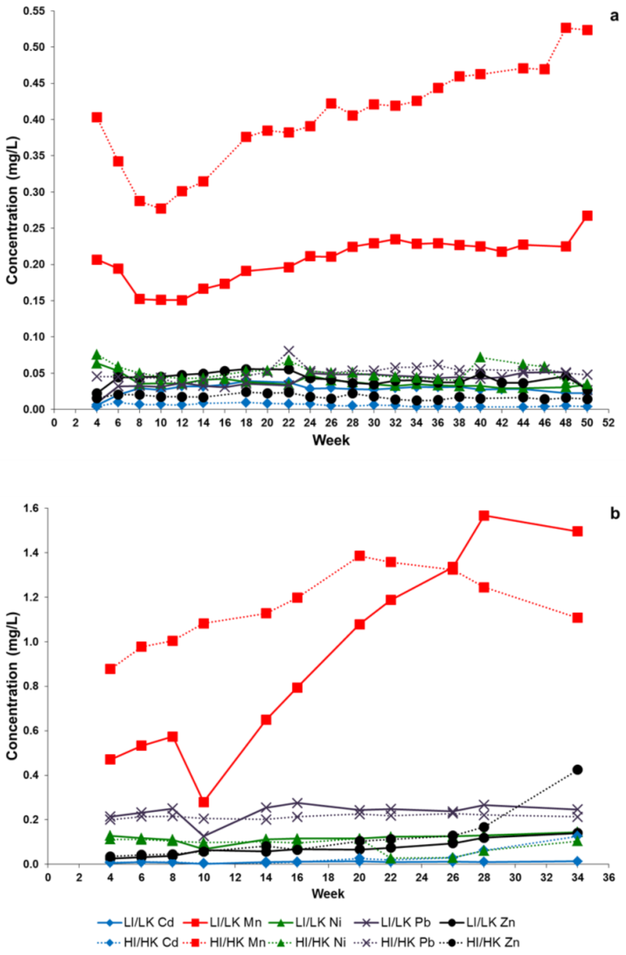

Figure 2.1 Effluent dissolved Cd, Mn, Ni, Pb, and Zn concentrations in LI and HI columns (a) and LK and HK columns (b)

Bulk and particle densities and porosity may vary a great deal from one VFBR substrate to another, with laboratory values ranging from 0.35 to 0.63 (Younger et al. 2002, Amos and Younger 2003, Neculita et al. 2008a). For the purposes of this study, bulk and particle density values were used to calculate the porosity of the SMC, in order to aid in final calculations of mass-loading and removal rates. Dry bulk density of the SMC was measured three separate times throughout the 30 day saturation period with a result of 0.177 ± 0.03 g/mL. The particle density of the

SMC was 1.37 ± 0.01 g/mL. Using the Equation 2.3, the resulting porosity of the SMC was 0.87 ± 0.02. This porosity is considerably greater than values found in the literature on VFBR, but is considered realistic for the material. It is worth noting that performing the calculation using the wet bulk density (0.791 ± 0.03 g/mL) results in a porosity of 0.42 ± 0.02. Both of these values are more consistent with the literature (Ginkel et al. 1999).

$$S_t = 1 - (\rho_b \div \rho_p) \tag{2.3}$$

Carbon

Initial SMC samples contained approximately 25 ± 0.3% total carbon, with an overwhelming majority of that (99 ± 1.7%) present as organic carbon. Carbon concentrations had increased by the end of the study, with LI and HI substrates containing 29 ± 1.2% and 29 ± 1.4% carbon, respectively. No significant differences in carbon fractions were found with regard to depth and all data within a given set of columns have been summarized together in Table 2.5. There was also no significant difference between the LI and HI substrates. Organic carbon made up 101 ± 4.2% of the total carbon in the LI substrates and 99 ± 8.6% of the total carbon in the HI substrates. Inorganic carbon concentrations were determined via difference between total and organic carbon and results were often negative. Despite this fact and the variability in calculated inorganic carbon concentrations, inorganic carbon data are presented to illustrate their very small contribution to the total carbon concentrations. Although dissolution of $CaCO_3$ was obvious, as evidenced by the increase in effluent Ca and alkalinity concentrations (Table 2.3), there was not a significant difference in inorganic carbon concentrations between SMC and LI and HI substrates and LK and HK substrates.

Table 2.5 Median total, organic, and inorganic carbon concentrations in all substrates

	SMC (n = 3)		**LI (n = 27)**		**HI (n = 27)**		**LK (n = 9)**		**HK (n = 9)**	
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**
Total C (g/kg)	253	5.5	287	14.7	287	26.1	315	22.0	303	9.8
Organic C (g/kg)	251	4.5	291	20.3	285	38.6	326	28.5	319	11.2
Inorganic C (g/kg)	3.8	7.8	-3.7	16.8	-1.8	20.1	-13.1	7.7	-22.1	10.4

Total metals

The original SMC contained 5.13 ± 0.09 g/kg Na, 100 ± 12 g/kg Ca, and low concentrations of Cd, Ni, and Pb (0.82 ± 0.04, 5.11 ± 0.5, and 6.85 ± 0.4 mg/kg, respectively). Elevated concentrations of Mn and Zn (298 ± 32 and 236 ± 31 mg/kg) were present in the SMC, but were not considered inhibitory to the processes required to remove these metals from SMD.

Calcium concentrations in all LI substrates were unchanged from the original SMC. They were, however, significantly higher in the top LI substrates than in the top HI substrates (75.9 ± 13.8 g/kg and 56.0 ± 4.1 g/kg, respectively), indicating enhanced dissolution of $CaCO_3$ in the HI columns. This effect was even greater in both the LK and HK columns, implying an added effect of the ammonium molybdate SRB inhibitor on the dissolution of $CaCO_3$. A significant amount of Na was flushed from the LI substrates, resulting in significantly lower concentrations (0.36 ± 0.01 g/kg) than the original SMC. A similar, but more extreme, pattern was seen in the LK column, with a final concentration of 0.18 ± 0.16 g/kg. Conversely, Na concentrations were significantly higher in the HI substrates, at 5.75 ± 0.89 g/kg, than in the SMC. The HK column also retained Na present in the SMC, with a final concentration of 4.91 ± 0.97 g/kg.

All spent substrate samples contained significantly higher concentrations of Cd, Mn, Ni, Pb, and Zn than the initial SMC, but there were no differences between LI and HI columns when examined as a whole. Total Mn concentrations were significantly greater in the LI (570 ± 101 mg/kg) and HI (550 ± 73 mg/kg) substrates than in the LK (180 ± 140 mg/kg) and HK (63 ± 109 mg/kg) substrates, reinforcing the differences in removal rates calculated from effluent concentrations (Table 2.4). The HK substrates contained significantly less Pb than the other three substrates. The LI, HI, LK, and HK substrates had Pb concentrations of 150 ± 153mg/kg, 107 ± 194 mg/kg, 101 ± 49 mg/kg, and, 24 ± 20 mg/kg, respectively.

There were significant differences in trace metals concentrations in the three sampling depths in substrates from all of the columns, resulting in a great deal of variability in the data. This variability is illustrated in the boxplots in Figure 2.2. At least 50% of the trace metals were removed in the top third of the LI columns, with median concentrations decreasing from the top to the bottom of the columns. Greater than 75% of Cd, Ni, and Pb were removed in the top third of the HI columns, with median concentrations again decreasing as depth increased. Overall, more Cd, Ni, Pb, and Zn were removed in the top third of the HI columns than the top third of the LI columns, while more Mn was removed in the top third of the LI columns. These relationships were reversed in the bottom of each set of columns with respect to Mn and Ni, with more Mn in the HI columns and more Ni in the LI columns.

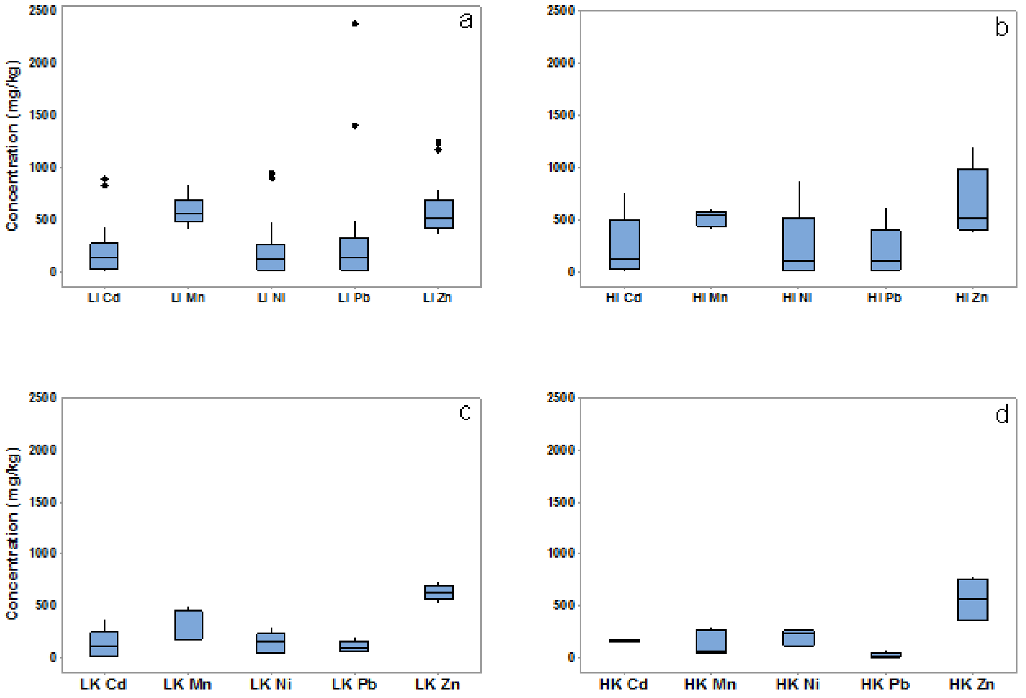

Figure 2.2 Boxplots of substrate Cd, Mn, Ni, Pb, and Zn concentrations in LI (a), HI (b), LK (c), and HK (d) columns (LI/HI n = 27; LK/HK n = 9)

As a whole, significantly more Mn was removed in LI and HI columns than in the LK and HK columns. In fact, Mn concentrations in the top two thirds of the LK substrates and all of the HK substrates were significantly lower than the original SMC, indicating a potential flushing or exchange of Mn in the initial substrate. There were no significant differences in concentrations of Cd, Ni, Pb, and Zn between the LI and LK substrates or in Cd, Ni, and Zn between HI and HK substrates. Significantly more Pb was found in HI substrates than in HK substrates.

AVS/SEM

The median AVS and SEM concentrations for the LI and HI column substrates are shown in Table 2.6. Trace metals contained in the substrate, but not bound up as amorphous sulfides, could also be susceptible to destruction by the hydrochloric acid used in the AVS/SEM extraction (Poot et al. 2009). This potentially results in SEM concentrations that exceed AVS concentrations. A SEM/AVS ratio > 1 indicates that at least some trace metals are retained in less stable, more bioavailable fractions, increasing toxicity of the substrate (Di Toro et al. 1992, Jong and Parry 2004). When SEM/AVS is < 1, sulfide released during the procedure is sufficient to account for all of the trace metals released. These metals are expected to be bound up as insoluble sulfides, decreasing potential toxicity of the substrate.

Table 2.6 Mean SEM (∑Cd, Mn, Ni, Pb, Zn) and AVS concentrations

	SEM (mmol/kg)	**AVS (mmol/kg)**	**SEM/AVS**
LI - top	39.4 ± 2.39	32.3 ± 6.77	1.28 ± 0.25
LI - middle	36.5 ± 3.14	33.3 ± 5.34	1.02 ± 0.16
LI - bottom	27.5 ± 2.89	38.1 ± 2.91	0.79 ± 0.03
HI - top	52.3 ± 6.62	51.5 ± 15.5	0.99 ± 0.20
HI - middle	39.0 ± 7.56	34.1 ± 14.5	1.15 ± 0.31
HI - bottom	27.4 ± 2.70	30.5 ± 4.58	0.90 ± 0.09

Although total SEM concentrations were greater in the top and middle of both sets of columns, the SEM/AVS ratios exhibited a large degree of variability and were often greater than one. This strongly indicates that although some Cd, Mn, Ni, Pb, and/or Zn may be removed as sulfides, they were most likely also removed via other mechanisms. However, SEM/AVS ratios were less than one in the bottom sections of both the LI and HI sets of columns, implying that the trace metals liberated during the extraction had been present as sulfides.

No sulfide was produced during extraction of any of the LK or HK substrate samples, but SEM concentrations ranged from ~22 mmol/kg in the tops of the columns to below detection limits (Table 2.1) in the bottoms of the columns. These results imply that although trace metals are being retained in the substrates, they are not being retained as sulfides. Consequently, these data are not included in Table 2.6.

Sequential extractions

The initial SMC contained very low total concentrations of Cd, Ni, and Pb and elevated concentrations of Mn and Zn (Table 2.7). Although Cd, Ni, and Pb concentrations were very low, it was evident that a large percentage of Cd was in the carbonate fraction and large percentages of Ni and Pb were in the labile organic fraction. Substantial percentages of these three metals were also in the refractory organic/sulfide fractions, but it was assumed that, given the oxic conditions of the material, they were associated with the refractory organic matter in the SMC. Roughly half of the Mn in the SMC was found in the carbonate fraction, most likely associated with the sugar beet lime ($CaCO_3$), and nearly a quarter was in the exchangeable fraction.

Table 2.7 Median (± ½ IQR) concentrations of Cd, Mn, Ni, Pb, and Zn in each operationally defined fraction in each set of columns and the original SMC

	SMC	**LI**	**HI**	**LK**	**HK**
Exchangeable					
Cd (mg/kg)	BDL	1.3 ± 1.2	1.2 ± 1.1	100 ± 98	110 ± 13
Mn (mg/kg)	51 ± 2.3	150 ± 62	130 ±15	120 ± 56	28 ± 45
Ni (mg/kg)	0.38 ± 0.01	0.73 ± 0.65	0.73 ± 0.91	20 ±17	17 ±14
Pb (mg/kg)	0.36 ± 0.29	1.4 ± 1.2	0.23 ± 0.43	<0.35	<0.35
Zn (mg/kg)	6.0 ± 0.20	1.2 ± 0.79	2.2 ± 1.2	190 ± 78	87 ± 14
Carbonate					
Cd (mg/kg)	0.042 ± 0.013	5.8 ± 6.6	11 ± 11	8.2 ± 5.7	14 ± 6.4
Mn (mg/kg)	120 ± 6.4	220 ± 35	245 ± 45	24 ± 42	43 ± 17
Ni (mg/kg)	0.38 ± 0.07	3.5 ± 6.6	6.6 ± 9.6	6.1 ± 2.2	8.9 ± 0.89
Pb (mg/kg)	0.45 ± 0.32	7.9 ± 12	3.9 ± 6.2	<0.06	<0.06
Zn (mg/kg)	39 ± 5.1	27 ± 12	34 ± 18	71 ± 20	38 ± 22
Labile Organic					
Cd (mg/kg)	<0.04	18 ± 18	43 ± 35	20 ± 15	39 ± 19
Mn (mg/kg)	19 ± 4.4	53 ± 5.8	65 ± 15	63 ± 13	3.7 ± 19
Ni (mg/kg)	1.2 ± 0.12	15 ± 21	32 ± 36	33 ± 15	53 ± 6.0
Pb (mg/kg)	1.9 ± 0.45	23 ± 22	22 ± 20	50 ± 20	0.35 ± 2.5
Zn (mg/kg)	220 ± 20	135 ± 34	184 ± 53	340 ± 73	500 ± 300
Oxide					
Cd (mg/kg)	<0.02	5.3 ± 5.6	4.0 ± 2.9	1.2 ± 0.62	1.7 ± 1.1
Mn (mg/kg)	26 ± 8.7	8.2 ± 2.1	11 ± 1.7	2.2 ± 0.92	1.5 ± 0.97
Ni (mg/kg)	<0.12	1.9 ± 4.1	4.2 ± 6.2	4.2 ± 3.0	10 ± 1.9
Pb (mg/kg)	0.28 ± 0.11	5.4 ± 6.7	3.0 ± 2.8	1.6 ± 0.52	0.54 ± 0.26
Zn (mg/kg)	13 ± 5.0	39 ± 12	46 ± 17	6.2 ± 1.4	7.3 ± 2.3
Refractory organic/sulfide					
Cd (mg/kg)	0.11 ± 0.004	110 ± 130	200 ± 220	2.6 ± 1.3	3.3 ± 3.5
Mn (mg/kg)	19 ± 2.2	44 ± 4.9	46 ± 5.1	22 ± 3.1	22 ± 9.4
Ni (mg/kg)	3.5 ± 0.38	110 ± 140	280 ± 260	90 ± 37	120 ± 12
Pb (mg/kg)	1.5 ± 0.08	120 ± 140	150 ± 140	11 ± 2.2	4.1 ± 1.8
Zn (mg/kg)	27 ± 4.3	450 ± 140	640 ± 270	17 ± 3.9	16 ± 10
Residual					
Cd (mg/kg)	0.0087 ± 0.0069	0.051 ± 0.072	0.10 ± 0.11	0.012 ± 0.004	0.012 ± 0.011
Mn (mg/kg)	1.4 ± 0.59	2.2 ± 0.27	2.4 ± 0.43	2.6 ± 0.43	2.3 ± 0.58
Ni (mg/kg)	0.12 ± 0.25	0.26 ± 0.062	0.10 ± 0.19	0.28 ± 0.10	0.20 ± 0.047
Pb (mg/kg)	0.15 ± 0.046	0.76 ± 0.40	0.72 ± 0.59	0.21 ± 0.058	0.12 ± 0.029
Zn (mg/kg)	0.85 ± 0.13	1.7 ± 0.28	2.2 ± 0.35	1.0 ± 0.17	1.3 ± 0.21

The vast majority of Cd was found in the refractory organic/sulfide fraction in LI (76.5 ± 2.5%) and HI (76.1 ± 8.0%) substrates (Figure 2.3a). The next largest fraction accounting for Cd removal in both sets of columns was the labile organic fraction, with 13.3 ± 3.4% in the LI substrates and 16.4 ± 6.5% in the HI columns. Although overall there was no significant difference between the LI and HI substrates in these fractions, significantly more Cd was removed in the labile organic fraction in middle and bottom HI substrates than in middle and bottom LI substrates, while significantly less was found in the refractory organic/sulfide fraction. As Cd concentration decreased through the columns, more was bound to humic and fulvic complexes in the HI substrates than in the LI substrates.

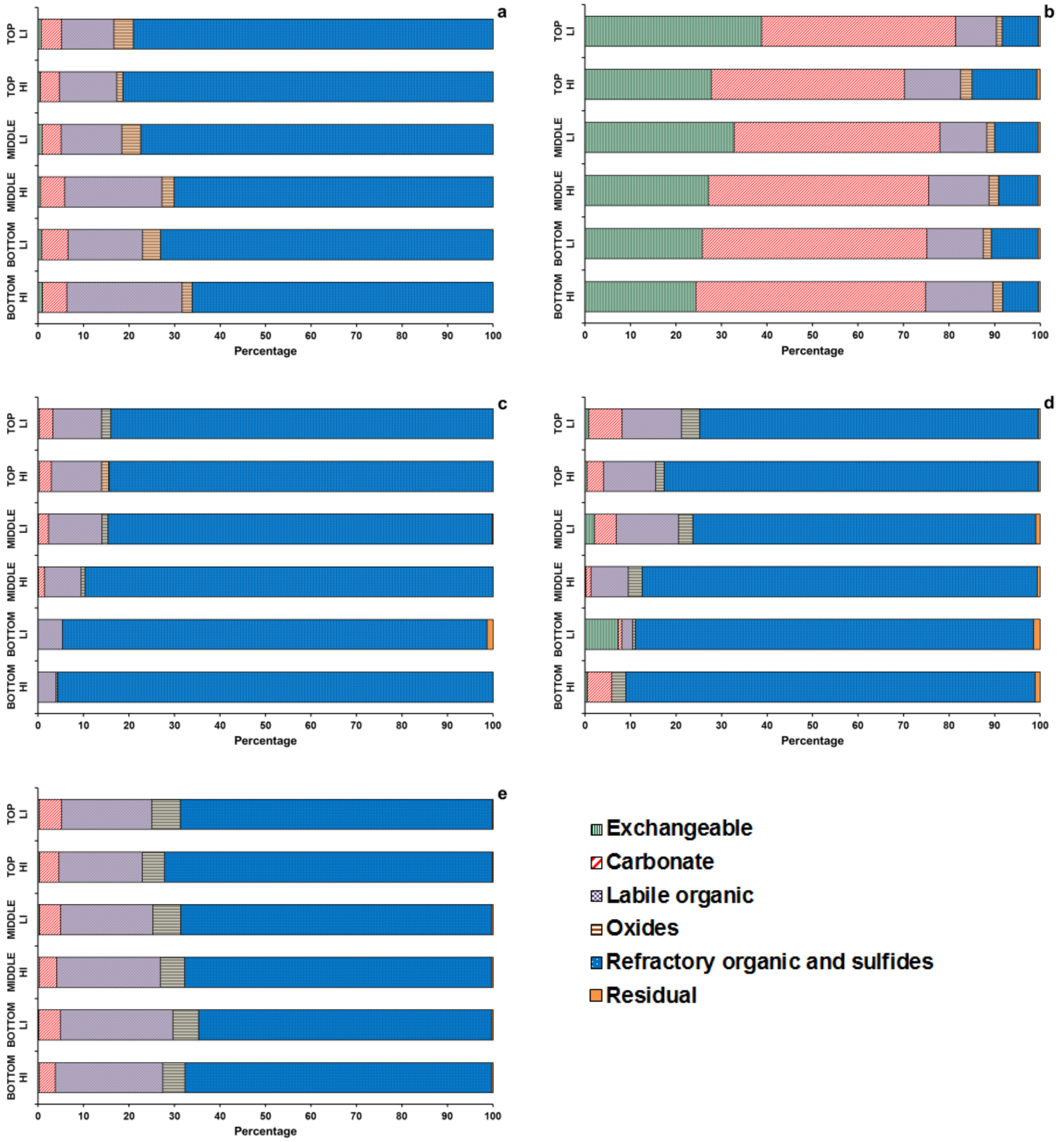

Figure 2.3 Sequential extraction fractions as percentages in top, middle, and bottom thirds of LI and HI substrates for a) Cd, b) Mn, c) Ni, d) Pb, e) Zn

No significant differences were found between total Cd concentrations in LI, HI, LK, and HK substrates, but there were significant differences in the removal products. The exchangeable fraction accounted for 77.2 ± 6.0% of the Cd in the LK substrate and 66.3 ± 15.3% in the HK substrate. Similar to the LI and HI columns, labile organic was the next largest fraction of Cd, with 12.9 ± 4.7% in LK substrates and, significantly more, 23.6 ± 4.4% in HK substrates.

The carbonate-bound fraction accounted for the majority of the Mn found in the substrates (Figure 2.3b). Although the concentration of Mn in this fraction was significantly higher in the LI and HI columns than in the SMC, the percentage of Mn in this fraction was unchanged from the initial substrate, with 46.1 ± 3.3%, 48.0 ± 3.6%, and 50.4 ± 0.9% in LI, HI, and SMC, respectively. Slightly more Mn was found in the exchangeable fraction in LI and HI substrates than in the SMC. In addition, the exchangeable fraction in LI substrates (31.0 ± 5.3%) was significantly greater than in HI substrates (26.4 ± 1.4%), indicating a small, but significant impact by ionic strength. This impact was most profound in the top third of the substrates where Mn concentrations were higher and pH was lower. Elevated ionic strength had an inhibitory effect on Mn adsorption in the HI substrates.

A higher percentage of Ni was found in the refractory organic/sulfide fraction than the other four trace metals examined (Figure 2.3c). This fraction accounted for 86.4 ± 3.4 % and 87.8 ± 5.3 % of Ni in LI and HI substrates, respectively. Approximately 10% was retained in the labile organic fraction in both sets of columns as well. Ionic strength did not seem to have an impact on how Ni was retained in the substrates as there were no significant differences in any fractions of Ni between LI and HI substrates. However, as total Ni concentration decreased from the top to the bottom of the columns, the percentage of Ni removed in the refractory organic/sulfide fraction increased significantly, while the percentages removed in the carbonate and labile organic fractions decreased.

More than half of the Ni in the LK and HK substrates was found in the refractory organic/sulfide fraction (61.2 ± 5.0% and 53.9 ± 5.2%, respectively), presumably bound to refractory organic material since no sulfide was present in the columns. Significantly more Ni was found in the labile organic fraction in the LK and HK columns than in the LI and HI columns, on both concentration and a percentage basis. Elimination of the potential for removal as a sulfide caused binding to organic matter as a whole to become the dominant removal process.

Lead was the only trace metal examined for which there was a difference in the refractory organic/sulfide fractions between the LI and HI columns, with 75.7 ± 6.6% and 83.9 ± 6.4% retained in that fraction (Figure 2.3d). In a manner similar to Ni, removal in this fraction increased in both sets of columns as the Pb concentration decreased through the columns. The second most abundant fraction, labile organic, decreased at the same time, for an overall 12.9 ± 7.5% and 10.6 ± 6.0% in the LI and HI columns, respectively.

Again similarly to Ni, elimination of sulfate reduction resulted in Pb being retained in association with organic matter as a whole. In the LK column, 74.6 ± 4.8% of the Pb was found in the labile organic fraction and 20.4 ± 4.1% was found in the refractory organic/sulfide fraction. In the HK column, these percentages were essentially reversed, with 21.2 ± 10.0% found in the labile organic fraction and 52.9 ± 8.9% in the refractory organic/sulfide fraction. The elevated ionic strength in the HK column resulted in much less Pb being associated with the labile organic fraction. Again, since no sulfide was found in these columns, removal as a sulfide was not considered viable.

There were no significant differences between LI and HI columns with regard to Zn retention (Figure 2.3e). The refractory organic/sulfide fraction accounted for 67.3 ± 2.4% of Zn retention in the LI columns and 69.2 ± 2.8% in the HI columns, while the labile organic fraction accounted for 20.3 ± 1.6% and 21.1 ± 2.4% in the LI and HI columns, respectively. Although there was a slight decrease in percentage of the former fraction and an increase in the latter fraction as Zn concentration decreased through the columns, ionic strength still appeared to have no impact on Zn removal.

Concentrations of Zn in both the exchangeable and labile organic fractions were significantly higher in the LK and HK columns than in the LI and HI columns. An increase in ionic strength was accompanied by an decrease in sorption of Zn to substrate surfaces, with 29.6 ± 14.3% and 11.9 ± 11.1% found in the exchanageable fraction in the LK and HK columns, respectively. Conversely, the amount of Zn retained in the labile organic fraction increased with ionic strength, from 55.4 ± 9.6% in the LK column to 77.8 ± 14.3% in the HK column.

Comparison of the concentrations in the refractory organic/sulfide fractions in the LI and HI substrates (Table 2.7) to SEM concentrations (Table 2.8) can aid in differentiating between concentrations associated with refractory organic materials versus those associated with sulfides. Concentrations of Cd and Pb were not significantly different between the two analyses, indicating that Cd and Pb found in the refractory organic/sulfide fraction were indeed present as sulfides.

Table 2.8 Concentrations of Cd, Mn, Ni, Pb, and Zn released during AVS/SEM extraction with hydrochloric acid

SEM (mg/kg)	**Cd**	**Mn**	**Ni**	**Pb**	**Zn**
LI	150 ± 96	480 ± 72	37 ± 29	130 ± 105	320 ± 63
HI	160 ± 230	460 ± 69	31 ± 49	120 ± 190	340 ± 220

There were significant differences between refractory organic/sulfide and SEM Mn, Ni, and Zn concentrations in both the LI and HI columns. Upon closer inspection, the SEM-Mn concentrations are closer to the total Mn concentrations than the refractory organic/sulfide fraction. Adsorption of Mn is tenuous (Bless et al. 2008) and $MnCO_3$ is readily soluble in cold dilute hydrochloric acid. This attempt to evaluate SEM-Mn concentrations likely released the exchangeable and carbonate fractions of Mn, resulting in an erroneous estimate of sulfide-bound Mn. In the case of SEM-Ni, the disparity between SEM and refractory/organic sulfide concentrations is due to the low solubility of NiS in cold, dilute hydrochloric acid (Simpson 2001, Jong and Parry 2004, Haynes 2015), resulting in an underestimation of sulfide-bound Ni. Considering that ZnS is readily soluble in the SEM extraction fluid, it must be inferred that the SEM results are the true measure of removal of Zn as a sulfide and the refractory organic/sulfide fraction truly consists of Zn bound to refractory organic matter and sulfide.

Although there were a few differences in abundance of specific removal products between LI and HI substrates, the overall trend in removal products was the same. In both LI and HI columns, Cd, Ni, Pb, and Zn removal products followed the trend of: refractory organic/sulfide > labile organic > carbonate > oxides > exchangeable > residual. The trend was different for Mn, but the same in LI and HI columns: carbonate > exchangeable > labile organic > refractory organic/sulfide > oxides > residual. In the absence of sulfate reduction in the LK and HK columns, retention of Ni, Pb, and Zn was dominated by association with the organic fractions in the substrates, followed by the carbonate and exchangeable fractions. On the other hand, Cd and Mn retention were dominated by the exchangeable fractions, followed by labile organic and carbonate fractions.

Mineralogy

To confirm that trace metals were actually retained within the substrate via either adsorption or precipitation, samples were analyzed with SEM-EDS. Upon initial analysis, it was quickly confirmed that comparitively low concentrations of removal products caused them to be diffuse and difficult to locate with the microscope. Substrate samples with the highest concentrations of trace metals were selected for analysis to increase the opportunity of locating removal products. The vast majority of images were comparable to that shown in Figure 2.4a, with a combination of sugar beet lime ($CaCO_3$) and SRB biofilm surrounded by the organic substrate. Individual metal-sulfides were rarely located, with most of the Cd, Ni, Pb, and Zn found being clustered together on organic surfaces with sulfur.

Bright areas on the organic matter in Figure 2.4b illustrate this clustering. Despite the difficulty in locating specific metal-sulfide associations, ZnS aggregates were identified in at least one sample (Figures 2.4c-d), confirming the existence at least of ZnS in the LI and HI columns.

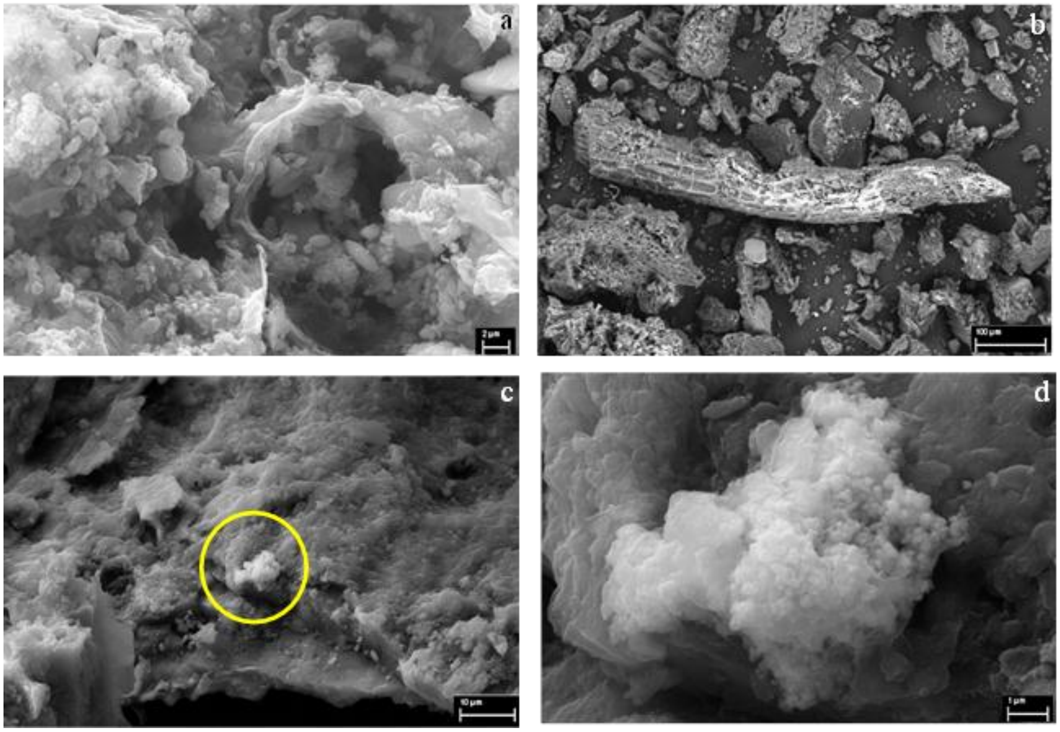

Figure 2.4 SEM-EDS micrographs of column substrates showing a) typical image made up of organic substrate, sugar beet lime ($CaCO_3$), and SRB biofilm (on the right half of the image), b) clustering of Cd, Pb, and Zn sulfides on substrate surfaces, c) ZnS aggregate on organic surface, d) magnification of area in yellow circle in (c). Note difference in scale in images.

Conclusions

Although there were significant differences in effluent trace metals concentrations between the LI and HI columns, due to heterogeneity of the substrate samples and much higher concentrations, there were no significant differences in total metals concentrations in the substrates. In instances where it was expected that ionic strength may have a strong impact on trace metal removal products, specifically the exchangeable and carbonate-bound fractions, the concentrations in those fractions were simply too low to compare effectively. However, Mn was an exception, with ionic strength having a negative impact on the amount removed through adsorption to the substrate. Ionic strength had the greatest impact on removal of Cd at the lower concentrations at the bottom of the columns, where the labile organic fraction became significantly higher in the HI columns and the refractory organic/sulfide fraction became higher in the LI columns. A similar situation occurred with Pb, but when concentrations were higher. Significantly more Pb was found in the labile organic fraction at the top of the LI columns and more was found in the refractory organic/sulfide fraction at the top of the HI columns. Although the results from the LK and HK columns were inconsistent in some places, they did serve to point out that VFBR may remove substantial amounts of trace metals in the absence of BSR. Removal of trace metals in these columns was dominated by adsortion to substrate surfaces and association with humic materials and refractory organic matter.

Although the SEM/AVS ratios indicated that a good portion of these metals may be bioavailable, these results must be observed with caution, considering the disagreement for Mn and Ni between SEM concentrations and refractory organic/sulfide fraction concentrations in the SEP. If the SEP paradigm that metal mobility and bioavailability decrease with each extraction, then stability of the trace metals decreases in the order of Ni > Cd > Pb > Zn >> Mn in the LI columns, Ni > Pb > Cd > Zn >>Mn in the HI columns, Ni > Pb > Zn > Mn > Cd in the LK column, and Ni > Pb > Zn > Cd > Mn in the HK column.

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Chapter Three: Characterization of mine drainage vertical flow bioreactor substrates using sequential extractions, acid-volatile sulfide analyses, and scanning electron microscopy

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Abstract

Passive treatment systems (PTS) have become a common tool in the treatment of mine drainage. Depending on source water quality and treatment needs, vertical flow bioreactors (VFBR) for the removal of trace metals may be an integral component of these systems. A PTS, including VFBR, was constructed in 2008 for the treatment of unabated net-alkaline ferruginous mine drainage in the Tar Creek Superfund Site in northeastern Oklahoma. Water quality data collected since the PTS began operation indicates significant removal of trace metals in the VFBR. Spent substrate samples from the VFBR were collected after 5.5 years of system operation and examined to determine the final fate of Cd, Co, Fe, Mn, Ni, Pb, and Zn within the VFBR. Results of a sequential extraction procedure (SEP) showed that the vast majority of Cd, Co, Fe, Ni, Pb, and Zn were retained in the refractory organic/sulfide fraction. Subsequent acid-volatile sulfide/simultaneously extracted metals (AVS-SEM) analyses confirmed the retention of Cd, Fe, Pb, and Zn as sulfides, but Co and Ni results were less certain due to the lack of solubility of Co- and Ni- sulfides in cold, dilute hydrochloric acid. Examination of the substrates via scanning electron microscopy (SEM) revealed an abundance of ZnS and FeS_2 aggregates attached to humic materials, with other trace metals often present in small concentrations. With the exception of Mn, trace metals were retained as insoluble products in the VFBR.

Introduction

Abandoned hard-rock mine sites are a widespread problem that present considerable ecological and human health risks (USEPA 2004, Limerick et al. 2005). Drainages from these sites are typically characterized by elevated metals (Al, Fe, trace metals) and sulfate concentrations, and will often be acidic (pH < 4.5) (Younger et al. 2002, Watzlaf et al. 2004). Conventional methods of treatment for mine drainages generally require large amounts of maintenance and can be very cost intensive to implement and sustain. Passive methods of treatment are becoming more commonplace. Passive treatment systems (PTS) utilize natural chemical, physical, and biological processes to remove contaminants from the water. These systems may be composed of a variety of unit processes, including anoxic limestone drains, vertical flow bioreactors (VFBR), and iron oxidation and settling ponds (Younger et al. 2002, Ziemkiewicz et al. 2003, Watzlaf et al. 2004)

Initially regarded as a process to produce anaerobic and reducing conditions in which to generate bicarbonate alkalinity through bacterial sulfate reduction (BSR) and limestone dissolution (Kepler and McCleary 1994), VFBR may also be used to remove trace metals and sulfate from mine drainage. Trace metal removal may occur through a variety of processes, including sorption to organic matter and precipitation as sulfides, hydroxides, and carbonates (Dvorak et al. 1992, Machemer and Wildeman 1992, Webb et al. 1998, Neculita et al. 2008). Considerable amounts of sulfate may also be removed through sorption and BSR, but the degree of removal depends strongly upon substrate composition, hydraulic retention time, and water quality (Dvorak et al. 1992, Cocos et al. 2002, Willow and Cohen 2003, Neculita et al. 2007, Pinto et al. 2015).

Of the potential trace metal removal processes in VFBR, precipitation as sulfides, facilitated by BSR, results in the most stable and least bioavailable product in the anoxic, reducing environment (Machemer and Wildeman 1992, Wildeman and Updegraff 1998, Song et al. 2001, Bless et al. 2008). Equation 3.1 illustrates the bacterial reduction of sulfate to produce soluble sulfide and bicarbonate and Equation 3.2 shows the reaction between the soluble sulfide, divalent trace metal, and bicarbonate to produce the desired insoluble metal-sulfide.

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^{-} + H_2S \qquad (3.1)$$

$$H_2S + M^{2+} + HCO_3^{-} \rightarrow MS\downarrow + 2H_2O + 2CO_2 \qquad (3.2)$$

Determination of the trace metal removal products can be made using a variety of procedures. Operationally-defined sequential extraction procedures (SEP) use a succession of selective chemical extraction fluids to release species associated with specific solid phases (Filgueiras et al. 2002, Gleyzes et al. 2002). The majority of these procedures place trace metals into five groups of species, with each one being more stable than the previous: i) adsorptive and exchangeable, ii) bound to carbonates, iii) bound to Fe/Mn oxides, iv) bound to organic matter and sulfides, and v) bound to primary and secondary minerals (Tessier et al 1979, Filgueiras et al. 2002, Gleyzes et al. 2002, Jong and Parry 2004). The addition of acid-volatile sulfide/simultaneously extracted metals (AVS/SEM) analyses may be used to augment the results of SEP, particularly the organic matter and sulfide fraction (Jong and Parry 2004).

The Tar Creek Superfund Site in northeastern Oklahoma continues to be one of the most demanding hard-rock mine reclamation sites in the US. The vast areal extent and substantial volume of contaminated ground and surface water have made treatment challenging at best (Nairn et al. 2001). The first documented discharges in the Tar Creek area after the cessation of mining occurred near southeast Commerce, OK (OWRB, 1983). These discharges contain elevated concentrations of Fe, Zn, Mn, Cd, Pb, and other trace metals, as well as sulfate, and account for approximately 20% of the contaminant mass loading in the watershed. After flowing unabated for nearly 30 years, passive treatment was determined to be a viable treatment option and a 10-cell treatment system, Mayer Ranch Passive Treatment System (MRPTS), was constructed and brought online in December 2008.

Methods and Materials

Site description

Construction and initial performance of MRPTS were described in detail in Nairn et al. (2009) and Nairn et al. (2010). The system was built to treat three mine discharges on the site with a total combined flow of 413 ± 55.7 L/min. Flow-weighted water quality data from the first sampling event in January 2009 through January 2015 are shown in Table 3.1.

Table 3.1 Flow-weighted median and first and third quartile influent mine drainage data at MRPTS, January 2009 – January 2015

	N	Q1	Median	Q3
Total flow (L/min)	30	349	413	460
T (°C)	33	17.8	17.8	17.9
pH (s.u.)	33	5.92	5.96	6.01
Cond (mS/cm)	33	2.82	3.00	3.05
DO (mg/L)	33	0.88	1.08	1.65
ORP (mV)	33	-39.0	109	132
Alkalinity (mg/L as $CaCO_3$)	33	381	397	407
Sulfate (mg/L)	24	2190	2280	2350
Ca (mg/L)	33	697	732	746
Mg (mg/L)	33	182	192	197
Na (mg/L)	33	94.3	101	105
Fe (mg/L)	33	168	173	181
Cd (mg/L)	33	0.011	0.016	0.023
Co (mg/L)	33	0.060	0.064	0.066
Mn (mg/L)	33	1.41	1.46	1.51
Ni (mg/L)	33	0.875	0.907	0.956
Pb (mg/L)	33	0.065	0.068	0.076
Zn (mg/L)	33	7.49	8.01	8.24

The three mine discharges at MRPTS flow into an initial oxidation pond, which flows into two parallel trains, each consisting of a surface-flow aerobic wetland, VFBR, re-aeration pond, and horizontal-flow limestone bed. The trains are then recombined in a final polishing wetland and discharged to the receiving stream. By the time the water reaches the VFBR that were the focus of this study, most of the Fe and some of the trace metals and alkalinity have been removed, although the water does remain net-alkaline.

Each of the two VFBR was constructed with an underdrain pipe system laid into limestone aggregate. This layer was covered by 0.5 m washed, high calcite limestone and 0.5 m reactive organic media. The media used in this PTS was a mix of 45% spent mushroom compost, itself made up of wheat straw amended with chicken litter, cottonseed meal, soybean meal, peat moss, sugar beet lime, and gypsum, 45% wood chips, and 10% manufactured limestone sand. The design surface area of the organic media was 760 m^2, for a combined surface area of 1520 m^2. Approximately 335 m^3 of organic media was placed in each cell, for a total volume of 670 m^3. Influent is allowed to flow freely into the VFBR and make its way downward through the organic media, limestone, and out through the underdrain system.

During placement of the organic media, a system for sampling porewater was also installed in each VFBR. Figure 3.1 shows the approximate dimensions of each VFBR and the locations of the porewater samplers. Seven porewater samplers were installed in each cell using ¼" (0.64 cm) LDPE tubing contained in 1" (2.5 cm) PVC pipe to protect the tubing from damage from the substrate and wildlife. The LDPE tubing was extended to the end of the PVC pipe in the organic media, which included a 71-cm long well screen (0.03-cm slot) to allow water to be sampled at that location within the media. The other end of the tubing and pipe was brought to the top of the VFBR, with enough tubing extending from the pipe to be connected to a peristaltic pump for porewater sampling.

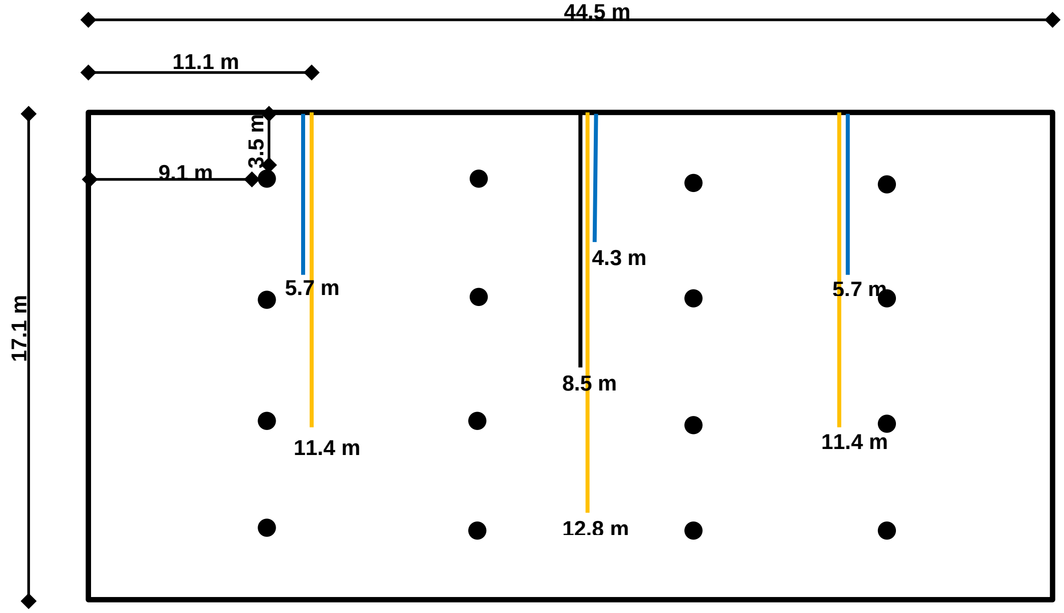

Figure 3.1 Dimensions of MRPTS VFBR and schematic of porewater sampling system (Black circles indicate sampling locations.)

Sampling and analysis

Water quality monitoring occurred at the VFBR beginning in January 2009 and continued through this study. In addition, porewater samples were collected in July 2014. Temperature, pH, conductivity, dissolved oxygen (DO), and oxidation-reduction potential (ORP) were measured with a YSI 600QS multiparameter datasonde and YSI 650MDS display. Total alkalinity was measured immediately after sample collection following Hach method 8203. Total metals and sulfate samples were collected during each sampling event in 250-mL HDPE bottles. Dissolved metals samples were collected approximately quarterly and filtered with 0.45-µm filters prior to preservation. Metals samples were preserved with trace metal grade HNO_3 to pH < 2 and sulfate samples were collected with no headspace and stored ≤ 4°C until analysis. When possible, flow was measured using a calibrated bucket and stopwatch. All metals samples were microwave digested with trace metal grade nitric acid following EPA method 3015 and analyzed for Ca, Mg, Na, Fe, Cd, Co, Mn, Ni, Pb, and Zn via ICP-OES according to EPA method 6010. Sulfate samples were analyzed via IC following EPA method 300.1 or via Hach DR3800 spectrophotometer according to Hach method 8051.

In addition to the regular water quality monitoring, samples were collected for carbon and sulfide analyses on three separate occasions in 2014-2015. Total and dissolved carbon samples were collected in 40-mL amber glass bottles with no headspace and stored ≤ 4°C until analysis. Dissolved carbon samples were filtered with 0.45-μm filters prior to preservation. All carbon samples were analyzed with an Analytik Jena N/C 2100 carbon analyzer following EPA method 9060. Sulfide samples were immediately preserved with sodium hydroxide and zinc acetate and analyzed according to APHA method 4500 D.

Additional porewater samples were collected in July 2014 and additional effluent samples were collected in July 2014 and January 2015 for determination of SRB populations. Bacteria culture bottles containing the American Petroleum Institute RP-38 sulfate reducer medium were obtained from VK Enterprises (Edmond, OK). Bottles were inoculated and serial diluted to 10^{-6} to determine populations. Samples were incubated at room temperature up to 28 days before determining the populations.

Substrate samples were collected from both VFBR in July 2014, 5.5 years after initial inundation. The number of samples to be collected was determined following the US EPA Guidance for Data Useability in Risk Assessment, Part A (1992), resulting in a sample size of 16 per VFBR. The substrate samples were collected at points on an equidistant grid in each VFBR. A drain spade was used to create a hole in the substrate to within 10 cm of the underlying limestone and a sample was collected by hand. Samples were immediately placed in a vacuum zipper bag, which was promptly sealed and evacuated with a FreshSaver® Handheld Vacuum System. The sealed bags were placed in second zipper bags, sealed, and vacuumed. All substrate samples were stored at ≤ 4° C until analyses were performed.

All substrate samples were returned to laboratories at the University of Oklahoma for analyses. All substrate analyses were performed in triplicate, for a total of 96 analyses per method. Moisture content was determined by drying samples at 60° C for 24 hours, after which time samples were weighed and retained for total metals, carbon, and mineralogical analyses. Subsamples of the dried substrate samples were predigested with nitric and hydrochloric acid for 12 hours under a fume hood prior to microwave digestion following EPA method 3051. All digested substrate samples were analyzed for Cd, Co, Fe, Mn, Ni, Pb, and Zn with a Varian Vista-PRO ICP-OES following EPA method 6010. Additional subsamples were analyzed for total, inorganic, and organic carbon content using an Analytik Jena N/C 2100 carbon analyzer following EPA method 9060.

Acid-volatile sulfide/simultaneously extracted metals (AVS/SEM) analyses were performed in triplicate on undried substrate samples. The analyses were conducted following Leonard et al. (1996), adapted from a modified diffusion method described in Brouwer and Murphy (1994). Resulting AVS samples were analyzed according to APHA method 4500 D and SEM samples were filtered using 0.45-μm polypropylene syringe filters and analyzed for Cd, Co, Fe, Mn, Ni, Pb, and Zn via ICP-OES.

A sequential extraction procedure (SEP) was used to determine trace metal removal products in undried substrate samples. The SEP consisted of six operationally defined fractions, including the exchangeable, carbonate, oxide, refractory organic/sulfide, and residual fractions from Tessier et al. (1979) and a labile organic fraction from Hall et al. (1996). In addition, in anticipation of low oxide concentrations due to the anoxic nature of the substrates, the Fe/Mn oxide fraction was shortened from six hours to one hour, according to Chester et al. (1988) and Arunachalam et al. (1996). The six fractions and the associated reagents and procedures are described in Table 3.2. Due to the abundance of carbonate and labile organic matter present in the substrate, the second and third fractions were extracted twice and combined.

Table 3.2 Descriptions and procedures for six-step sequential extraction procedure

Fraction	Target	Reagents and procedure
Exchangeable	Metals that may be released through ion-exchange processes or are weakly adsorbed to the substrate surface	8-mL 1 M $MgCl_2$ at pH 7 Agitated for 1 hour
Bound to carbonate	Metals that are precipitated or co-precipitated with carbonate and metals that are adsorbed to carbonate surfaces	20-mL 1 M NaOAc adjusted to pH 5 with HOAc Agitated for 1 hour and repeated
Bound to labile organic matter	Metals that are bound in humic and fulvic acids through complexation	30-mL 0.1 M $Na_4P_2O_7 \cdot 10H_2O$ at pH 10 Agitated for 1 hour and repeated
Bound to Fe/Mn oxides	Fe and Mn oxides and any metals that may be adsorbed to them	20-mL of 0.04 M $NH_2OH \cdot HCl$ in 25% (v/v) HOAc Agitated for 1 hour
Bound to refractory organic matter and sulfides	Metals that are bound to sulfides and decay-resistant organic matter with low solubility	3-mL of 0.02 M HNO_3 and 5-mL 30% H_2O_2 adjusted to pH 2 with HNO_3 Heated to 85±2°C for 2 hours with occasional agitation 3-mL 30% H_2O_2 Heated to 85±2°C for 3 more hours with occasional agitation After cooling, 5-mL of 3.2 M NH_4OAc in 20% (v/v) HNO_3 and 4-mL sparged ultrapure water Agitated for 30 min
Residual	Metals that are bound to primary and secondary minerals, particularly silicates, which typically enter the environment through weathering	10-mL concentrated HNO_3 Microwave digestion

All extraction fluids were made with nitrogen-sparged ultrapure water in order to prevent oxidation of the substrates. The SEP was performed in triplicate using approximately 1 g (dry weight) of undried sample. Extractions were carried out in a nitrogen-filled anaerobic chamber to prevent the alteration of removal products via oxidation. After each extraction was performed, samples were centrifuged at 10000 rpm for 15 minutes and the supernatant collected in 60-mL HDPE bottles. Residues from the first through fifth steps were rinsed with 10-mL nitrogen-sparged ultrapure water and centrifuged for 15 minutes, after which time the rinse was collected and added to the supernatant. All SEP samples were filtered with 0.45-μm polypropylene filters and analyzed for Cd, Co, Fe, Mn, Ni, Pb, and Zn with ICP-OES.

Dried samples were prepared for mineralogical analyses by mounting on Al stubs with carbon tape and sputter-coating with Au-Pd alloy. Analysis was conducted with a Zeiss NEON 40 EsB field emission scanning electron microscope fitted with an INCA Energy 250 energy dispersive x-ray microanalysis system (SEM-EDS) and an Oxford backscatter detector.

Statistics

All statistics were calculated using the statistical software package Minitab v.17.2.1 (Minitab 2010). A significance level of 0.05 was applied to all calculations. The Mann-Whitney test was used to determine significant differences between two sets of data.

Results and Discussion

Water quality

Over the course of the 5.5 years between inundation of the VFBR and sampling of the substrates, water surface levels fluctuated a great deal due to flow constriction in the substrate. The original surface dimensions of the organic media in the VFBR, measured during installation of the porewater sampling system, were approximately 44.5 m x 17.1 m. However, when surface dimensions were measured during substrate sampling in July 2014, they were approximately 41.0 m x 14.8 m. Although some settling of the material was expected after inundation, a decrease of ~20% was unexpected. Based upon laboratory examination, settling and compaction was believed to have occurred within approximately 30 days after inundation.

Due to the design of the system, it is not possible to measure volumetric flow rates leaving the VFBR. In addition, due to elevated water levels due to the aforementioned flow constriction, it eventually became impossible to measure flow rates into the VFBR as well. On only five occasions were flows into both VFBR able to be measured during the same sampling event. These flow rates, presented in Table 3.3, and the newly measured surface dimensions were used for all loading and removal rate calculations.

Table 3.3 Combined median and first and third quartile concentrations in the influent, effluent, and porewater of MRPTS VFBR

		Influent (Jan 2009- Jan 2015)				**Effluent (Jan 2009-Jan 2015)**				**Porewater (July 2014)**			
		N	**Q1**	**Median**	**Q3**	**N**	**Q1**	**Median**	**Q3**	**N**	**Q1**	**Median**	**Q3**
Flow (L/min)		5	289	309	325	-	-	-	-	-	-	-	-
T (°C)		66	9.10	19.1	25.9	66	6.76	17.8	23.8	14	27.2	28.3	28.6
pH (s.u.)		66	6.24	6.39	6.69	66	6.67	6.79	6.88	14	6.68	6.75	6.79
Cond (mS/cm)		66	2.27	2.80	3.18	66	2.16	2.60	3.10	14	3.42	3.50	3.57
DO (mg/L)		66	6.30	8.55	10.8	66	0.210	0.750	1.89	14	1.17	1.34	1.50
ORP (mV)		66	105	180	279	66	-204	-90.5	41.3	14	-124	-82.3	-13.0
Alk. (mg/L as $CaCO_3$)		66	134	145	160	66	179	220	273	14	139	160	171
Sulfate (mg/L)		52	2110	2250	2390	52	2040	2140	2280	14	2030	2080	2100
Sulfide (mg/L)		0	-	-	-	6	1.27	3.21	9.25	14	0.298	0.715	0.996
Total C (mg/L)	**Tot**	6	32.9	40.9	43.4	6	37.1	52.7	63.6	14	39.9	43.0	45.5
	Diss	6	33.6	38.9	42.6	6	37.8	53.1	66.9	14	40.1	43.9	48.6
Organic C (mg/L)	**Tot**	6	1.31	1.68	2.20	6	0.28	1.21	1.50	14	1.55	1.70	2.29
	Diss	6	1.91	2.07	2.40	6	0.35	1.49	1.78	14	1.60	1.82	1.94
Inorganic C (mg/L)	**Tot**	6	31.7	38.9	41.5	6	35.7	50.3	63.7	14	38.2	40.9	43.8
	Diss	6	31.0	37.1	40.4	6	36.0	50.5	66.1	14	38.4	42.0	46.6
Ca (mg/L)	**Tot**	66	698	720	748	66	705	749	774	14	701	709	718
	Diss	36	685	699	760	36	689	716	783	14	717	721	725
Mg (mg/L)	**Tot**	66	178	192	200	66	175	193	203	14	167	170	173
	Diss	36	175	183	191	36	172	183	190	14	169	170	173
Na (mg/L)	**Tot**	66	93.1	100	108	66	92.3	101	109	14	90.5	91.0	91.2
	Diss	36	91.7	102	106	36	89.8	102	107	14	90.7	91.3	91.7
Fe (mg/L)	**Tot**	66	2.19	3.51	7.54	66	0.313	0.725	1.91	14	1.12	1.55	2.10
	Diss	35	0.060	0.310	6.02	34	0.113	0.418	1.23	14	0.494	1.29	2.12
Cd (mg/L)	**Tot**	26	0.001	0.001	0.002	10	0.001	0.001	0.001	6	0.001	0.001	0.001
	Diss	10	0.001	0.002	0.003	6	0.001	0.001	0.001	5	0.001	0.001	0.001
Co (mg/L)	**Tot**	66	0.038	0.056	0.065	38	0.003	0.008	0.020	10	0.002	0.008	0.023
	Diss	36	0.032	0.051	0.067	17	0.003	0.009	0.012	10	0.002	0.006	0.022
Mn (mg/L)	**Tot**	66	1.20	1.44	1.61	66	0.740	1.03	1.59	14	1.39	1.81	2.69
	Diss	36	1.10	1.36	1.75	36	0.669	1.21	2.10	14	1.35	1.80	2.68
Ni (mg/L)	**Tot**	66	0.709	0.790	0.831	66	0.039	0.113	0.289	14	0.100	0.161	0.296
	Diss	36	0.716	0.758	0.804	35	0.043	0.097	0.266	14	0.094	0.152	0.286
Pb (mg/L)	**Tot**	5	0.026	0.029	0.031	1	0.029	0.029	0.029	0	-	-	-
	Diss	6	0.026	0.027	0.029	3	0.025	0.030	0.028	0	-	-	-
Zn (mg/L)	**Tot**	66	4.24	5.26	6.51	66	0.139	0.577	1.686	14	0.060	0.525	1.27
	Diss	36	3.82	4.83	5.42	35	0.009	0.019	0.177	14	0.046	0.162	1.15

After going through the oxidation pond and surface-flow aerobic wetlands, the mine drainage gained a significant amount of DO and lost a significant amount of Fe and alkalinity through oxidation, hydrolysis, and precipitation of Fe-oxyhydroxides. In addition, a significant amount of trace metals Cd, Ni, Pb, and Zn were removed via adsorption to and/or co-precipitation with the Fe solids. The water entering the VFBR remained net-alkaline with a circumneutral pH, but with significantly lower concentrations of trace metals to remove.

The anoxic, reducing conditions required for BSR were present in the VFBR, with pH in the 5-8 range considered ideal for SRB productivity and removal of trace metals as sulfides (Postgate 1984, Elliot et al. 1998, Willow and Cohen 2003, Tsukamoto et al. 2004). Populations of SRB were well established in the VFBR, with an average concentration of 10^3-10^4 organisms/cc in the porewater samples collected in July 2014. Although SRB population within the substrate solids was not determined, it has been found that it can be up to two orders of magnitude higher than the population in the aqueous phase (Zaluski et al. 2003). Removal of sulfate and production of sulfide were also strong indicators of the presence and activity of SRB. Populations in the effluent may have been influenced by season, with an average concentration of 10^3 organisms/cc in July 2014 and, slightly lower, 10^2-10^3 organisms/cc in January 2015.

Sulfate reduction played a significant role in raising the pH in the VFBR, as demonstrated in the porewater samples. A lack of significant increase in alkalinity, inorganic carbon, and Ca in the porewater samples indicated that dissolution of the manufactured limestone sand was not a significant factor in increasing the pH within the organic layer of the VFBR. Alkalinity concentrations did not increase significantly until the water had passed through the underlying limestone layer and were measured in the effluent.

Dissolved Fe increased in the porewater samples, presumably due to reduction of residual Fe-oxyhydroxides that did not settle in the aerobic wetlands and made their way into the VFBR. However, once the Fe was resolubilized, it was removed through reductive processes. Effluent Fe concentrations were significantly lower than influent Fe concentrations.

Both Co and Ni had been mostly removed in the porewater samples. The majorities of both metals were in the dissolved form, indicating that the trace metal removal products had been retained in the substrate. Concentrations of both Co and Ni decreased slightly in the effluent, likely due to continued sulfide production through the remainder of the organic media. The majority of Cd and Pb in the mine drainage are removed prior to the VFBR. Although it is difficult to evaluate any actual decrease in concentration, both metals were detected in the effluent in fewer sampling events than in the influent. Of the 14 porewater samples collected, Cd was found in only 6 and Pb was not found in any.

Although a substantial amount of Zn is removed from the mine drainage via adsorption and/or coprecipitation in the oxidation pond and aerobic wetlands, significantly more is removed in the VFBR. The vast majority of Zn removed in the VFBR is removed in the organic substrate, as evidenced by the significant decrease in porewater Zn concentrations. Dissolved Zn is further removed beyond this point, with even lower concentrations in the effluent. Whether this further removal occurs in the organic media or through exchange processes in the underlying limestone layer is unknown.

Elevated concentrations in porewater, in comparison to both the influent and effluent, indicate Mn may be desorbing from the substrate. Although lower median Mn concentrations in the effluent demonstrate that Mn is being removed overall, it is possible that it is removed as $MnCO_3$ in the limestone layer underlying the organic media layer, rather than in the organic layer itself. Although the effluent does not exhibit the $pH > 8$ required for $MnCO_3$ formation (Zagury et al. 2006, Neculita et al. 2007), microenvironments within the limestone layer may exhibit the elevated pH required for $MnCO_3$ precipitation.

Overall, the VFBR were effective at removing the majority of Co, Fe, Ni, and Zn and virtually all of the Cd and Pb (Table 3.4). Sulfate and Mn removal rates were considerably lower. Given the tendency for Mn to have weak sorption and sulfide formation capabilities and the fact that $pH < 8$ in the VFBR, a great deal of Mn removal was not expected (Dvorak et al. 1992, Zaluski et al. 2003, Willow and Cohen 2003, Zagury et al. 2006). Although the percent removal of sulfate seems low, sulfate removal rates vary widely in the literature (Neculita et al. 2007).

Table 3.4 Area-adjusted loading and removal rates in the MRPTS VFBR

	Loading rate ($g/m^2/d$)	**Removal rate ($g/m^2/d$)**	**% removal**
Sulfate	1653	81.4	4.92
Cd	0.0009	0.0009	99.9
Co	0.041	0.035	85.7
Fe	2.57	2.04	79.3
Mn	1.06	0.30	28.5
Ni	0.58	0.50	85.7
Pb	0.021	0.021	99.9
Zn	3.86	3.43	89.0

Substrates

Concentrations of organic and inorganic carbon varied widely due to the difficulty in completely mixing the three components of the organic media, resulting in a considerable amount of heterogeneity (Table 3.5). Despite this fact, a couple of tendencies could be discerned. Organic carbon concentrations in the spent substrates collected in July 2014 were elevated above concentrations in the original SMC. Although it was not significant, an increase in organic carbon concentrations could indicate either a contribution from the microbial growth or, more likely, a contribution from decomposed vegetation from the water column overlying the substrate. Inorganic carbon concentrations were found to have decreased in the substrates over time. Considering that aqueous inorganic carbon concentrations in the porewaters were not significantly higher than in the influent, the decrease in inorganic carbon in the spent substrates indicates that the manufactured limestone sand has either dissolved during system operation or has been flushed into underlying layers.

Table 3.5 Combined median and first and third quartile carbon concentrations in pre-construction (2008, n = 6) and spent substrates (2014, n = 96)

	November 2008			**July 2014**		
	Q1	**Median**	**Q3**	**Q1**	**Median**	**Q3**
Total carbon (g/kg)	139	162	182	129	141	152
Organic carbon (g/kg)	54.1	78.1	87.0	58.2	81.9	109
Inorganic carbon (g/kg)	60.1	82.1	104	32.7	53.4	75.7

Substrate samples collected during construction of the PTS and before inundation of the system contained elevated concentrations of Fe and much smaller concentrations of Cd, Co, Mn, Ni, Pb, and Zn. Table 3.6 shows the total metals concentrations of both the initial substrate from November 2008 and the spent substrates collected in July 2014. Concentrations of Co, Fe, Ni, Pb, and Zn increased significantly in the spent substrates. The absence of significant change in the Cd concentrations was due to the majority being removed from the mine drainage through co-precipitation and/or sorption to iron oxyhydroxides before it reached the VFBR. Lack of significant change in Mn concentrations in the substrate reinforces the idea that any Mn removal that occurs, may occur in the underlying limestone layer.

Table 3.6 Median and first and third quartile total metals concentrations in pre-construction substrates (2008, n = 6) and spent substrates (2014, n = 96)

	November 2008			**July 2014**		
	Q1	**Median**	**Q3**	**Q1**	**Median**	**Q3**
Fe (g/kg)	4.33	5.50	5.89	7.08	9.71	12.8
Zn (g/kg)	0.072	0.093	0.098	9.40	13.5	21.5
Cd (mg/kg)	0.985	1.14	1.26	0.916	1.16	1.43
Co (mg/kg)	-	-	-	49.5	70.9	136
Mn (mg/kg)	274	313	338	279	347	420
Ni (mg/kg)	4.62	5.53	5.96	1140	1560	2570
Pb (mg/kg)	11.5	14.1	14.4	15.1	17.5	21.8

In general, SEP concentrations were greater in the spent substrates than in the pre-construction substrates (Table 3.7). Notable exceptions are the Fe and Mn concentrations in the carbonate, labile organic, oxide, and residual fractions. Concentrations of both of these metals in these fractions did not change significantly over the 5.5 years the VFBR had been operating when the substrates were collected.

In addition to the actual concentrations, results of the SEP presented as percentages of the sum total are illustrated in Figure 3.2. With the exception of Mn, removal of trace metals in the exchangeable, carbonate, and oxide fractions is negligible in comparison to other fractions. Although Pb had the highest labile organic percentage of the seven trace metals evaluated, the concentration was not significantly different from the original substrate. Cobalt, Ni, and Zn concentrations in the labile organic fraction did increase significantly from the original substrate, but this did not make a large contribution to the overall removal of those metals.

Table 3.7 Median (± ½ interquartile range) concentrations (mg/kg) for each SEP fraction in pre-construction substrates (2008, n = 6) and spent substrates (2014, n = 96)

	November 2008	July 2014		November 2008	July 2014
Exchangeable			**Oxides**		
Cd	<0.01	<0.01	**Cd**	0.08 ± 0.01	0.041 ± 0.024
Co	<0.02	0.019 ± 0.19	**Co**	<0.03	1.31 ± 0.93
Fe	0.89 ± 0.28	<0.01	**Fe**	475 ± 32.7	407 ± 196
Mn	30.6 ± 4.12	60.3 ± 26.3	**Mn**	38.3 ± 6.96	25.9 ± 9.49
Ni	<0.07	5.01 ± 3.56	**Ni**	0.30 ± 0.02	46.0 ± 16.8
Pb	0.17 ± 0.10	<0.35	**Pb**	0.22 ± 0.08	0.58 ± 0.086
Zn	0.21 ± 0.15	2.54 ± 1.13	**Zn**	3.75 ± 0.51	358 ± 156
Carbonate			**Refractory organic/sulfide**		
Cd	0.04 ± 0.00	<0.03	**Cd**	0.36 ± 0.02	0.83 ± 0.19
Co	<0.05	1.17 ± 1.00	**Co**	0.74 ± 0.08	60.3 ± 38.6
Fe	131 ± 18.9	109 ± 88.7	**Fe**	746 ± 90.3	5605 ± 1360
Mn	92.3 ± 9.18	92.2 ± 26.3	**Mn**	25.0 ± 3.74	62.4 ± 15.7
Ni	<0.20	48.1 ± 32.4	**Ni**	2.75 ± 0.22	1140 ± 495
Pb	0.46 ± 0.26	<0.97	**Pb**	3.55 ± 0.42	8.16 ± 1.74
Zn	14.62 ± 1.14	134 ± 56.9	**Zn**	10.5 ± 1.44	16100 ± 6130
Labile organic			**Residual**		
Cd	<0.04	0.019 ± 0.023	**Cd**	0.02 ± 0.00	0.03 ± 0.014
Co	<0.07	4.36 ± 1.74	**Co**	<0.01	0.69 ± 0.65
Fe	1410 ± 254	944 ± 457	**Fe**	256 ± 29.7	320 ± 126
Mn	21.1 ± 2.36	18.8 ± 4.87	**Mn**	1.65 ± 0.36	2.12 ± 0.61
Ni	1.15 ± 0.07	109 ± 33.3	**Ni**	0.25 ± 0.03	11.8 ± 25.7
Pb	2.46 ± 0.39	1.36 ± 0.67	**Pb**	0.29 ± 0.03	0.34 ± 0.12
Zn	37.3 ± 4.23	547 ± 108	**Zn**	1.07 ± 0.16	97.7 ± 174

The greatest percentage of Mn was found in the carbonate-bound fraction, followed by the exchangeable fraction. Given that the conditions were not appropriate for actual $MnCO_3$ formation and that Mn concentrations in the spent substrates were not significantly different from the initial substrates, it was believed that this fraction was included in the limestone sand that was a part of the original mix. Any Mn that was found above concentrations in the initial substrate was found in the exchangeable and sulfide phases. Given that MnS was not expected to form due its high solubility and low aqueous concentrations of Mn, the Mn found in the refractory organic/sulfide fraction was believed to be associated with refractory organic material.

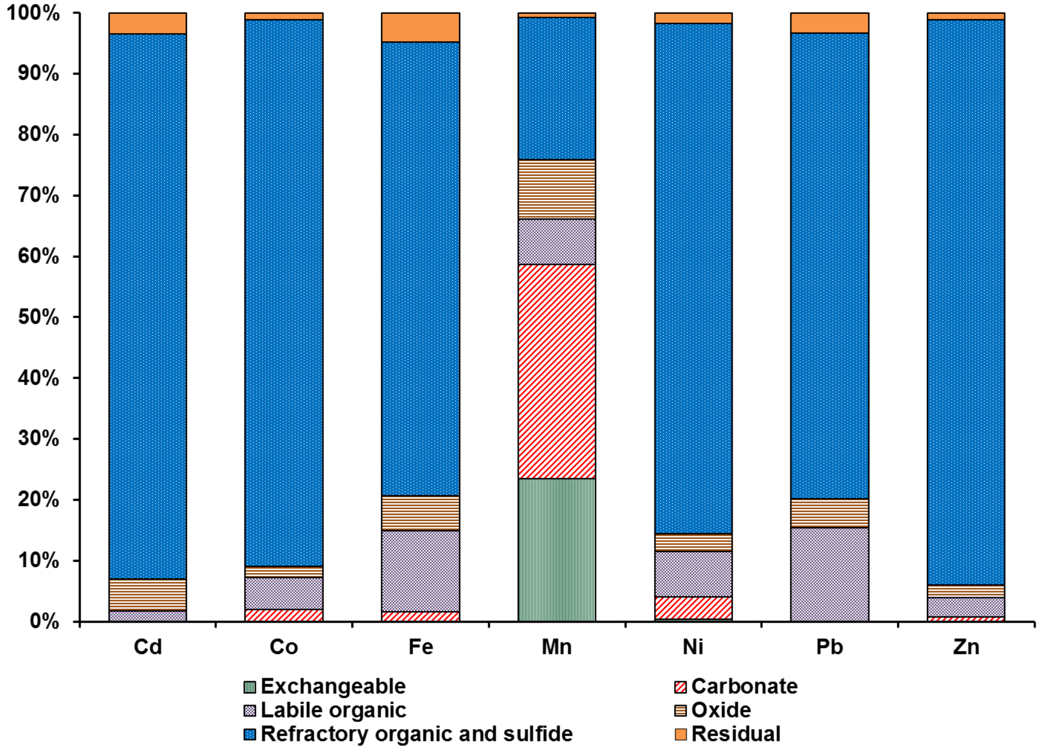

Figure 3.2 Median fractions of trace metals in VFBR substrates (n = 96). Results are presented as percentages

Very high percentages of Cd, Co, Fe, Ni, Pb, and Zn were found in the refractory organic/sulfide fraction (87.6%, 90.1%, 73.1%, 83.3%, 76.2%, and 92.3%, respectively). In order to estimate how much of this fraction was made up of sulfides, the results from the SEP were compared to the results from the AVS/SEM analyses. Concentrations of SEM and refractory organic/sulfide are shown in Table 3.8.

Table 3.8 Comparison of concentrations found in SEM and refractory organic/sulfide fractions (n = 96)

	SEM	**Refractory organic and sulfide**
Cd (mg/kg)	0.53 ± 0.13	0.83 ± 0.18
Co (mg/kg)	25.1 ± 11.9	60.3 ± 37.8
Fe (g/kg)	2.81 ± 0.70	5.60 ± 1.31
Mn (mg/kg)	304 ± 71.6	62.4 ± 15.6
Ni (mg/kg)	299 ± 81.4	1140 ± 485
Pb (mg/kg)	6.45 ± 1.24	8.30 ± 1.73
Zn (g/kg)	19.2 ± 9.4	16.1 ± 6.07

AVS/SEM analyses resulted in AVS concentration of 288.5 ± 81.3 mmol/kg and SEM (∑Cd, Co, Fe, Mn, Ni, Pb, Zn) concentration of 207.6 ± 150.4 mmol/kg. The resulting SEM/AVS ratio was 1.12 ± 0.20. The implication of SEM/AVS > 1 is that some trace metals, rather than being bound up as sulfides, will be bound up in more soluble and bioavailable products (Jong and Parry 2004). This is confirmed in the SEP results.

Concentrations of Cd, Co, Fe, Ni, and Pb were significantly higher in the refractory organic/sulfide fraction than in the SEM. Sulfides of Co and Ni are only slightly soluble in cold, dilute hydrochloric acid, resulting in lower concentrations in the SEM (Simpson 2001, Jong and Parry 2004, Haynes 2015). In the case of Cd and Pb, it is more likely that the SEM concentration is more indicative of the amount of these trace metals removed as sulfides. Both of these metals form strong relationships with organic matter and were likely partially present in the refractory organic fraction.

The difference in refractory organic/sulfide and SEM Fe concentrations is most likely due to the presence of pyrite. Pyrite is not readily soluble in cold, dilute hydrochloric acid, but would be oxidized by the heated, acidic H_2O_2, resulting in a higher concentration being found in the refractory organic/sulfide fraction (Rickard and Morse 2005).

Analyses with SEM-EDS revealed an abundance of Fe- and Zn- sulfide precipitates. When the less abundant trace metals were occasionally found under the microscope, they were primarily associated with the ZnS and surrounding organic substrate (Figure 3.3a). In other cases, nearly pure ZnS particles were found aggregated around organic substrate, as shown in Figure 3.3b. Although AVS/SEM analyses indicated that Fe-monosulfides were present in the substrates, FeS_2 was identified more frequently with SEM-EDS than Fe-monosulfides. Although much of the FeS_2 was found attached to humic materials (Figure 3.3c), several instances of framboidal pyrite were encountered (Figure 3.3d).

Conclusions

The VFBR at MRPTS retained the vast majority of trace metals that passed through it in the 5.5 years of operation before substrates were collected. Influent and effluent water quality analyses show that at least some Mn is being removed from the mine drainage, but substrate analyses did not bear this out well. It is believed that a continual cycle of sorption and desorption is occurring, resulting in highly variable porewater and effluent Mn concentrations. Although in some cases trace metals concentrations were sufficient for precipitation as carbonates, the circumneutral pH prevented their formation. The majority of Cd, Co, Fe, Ni, Pb, and Zn were removed in the refractory organic/sulfide fraction of a SEP, with subsequent AVS/SEM analyses further confirming their removal as sulfides. If percentage retained in the refractory organic/sulfide fraction is an indicator of stability of trace metal removal products, then stability of trace metals in the VFBR are ranked in the order of Zn > Co > Cd > Ni > Pb > Fe >> Mn. Overall, with the exception of Mn, trace metals were retained in the VFBR in a form that will remain stable and biologically unavailable in the anoxic, reducing environment.

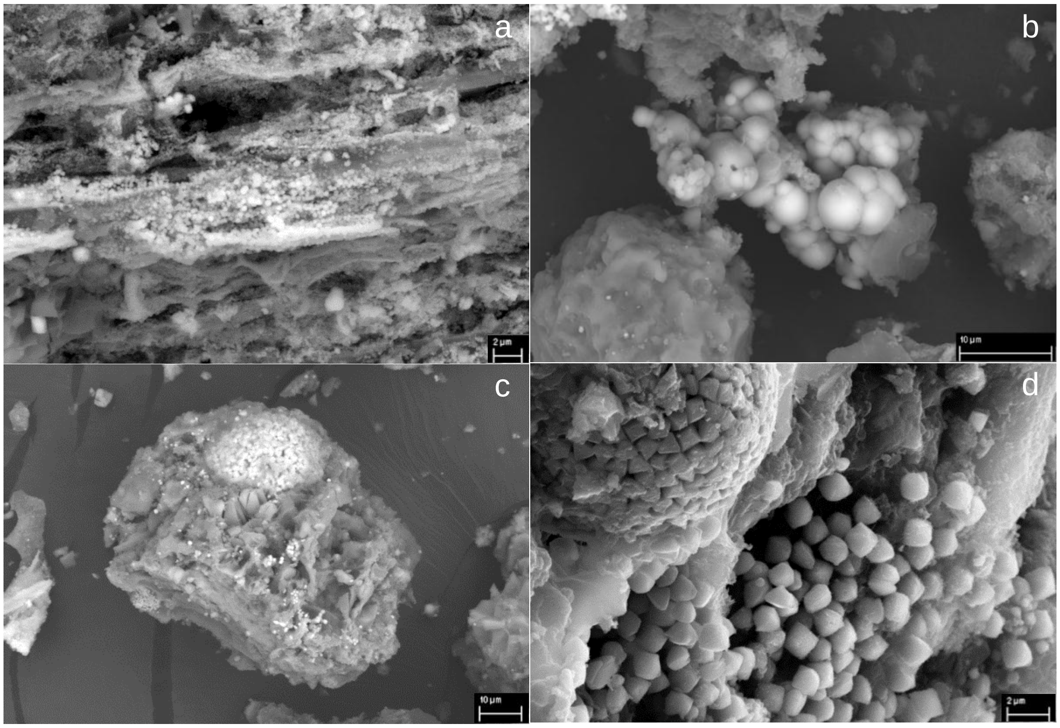

Figure 3.3 SEM-EDS micrographs of VFBR substrates showing a) ZnS aggregation on humic material, b) more developed ZnS colloidal aggregates, c) FeS_2 aggregation on humic material, d) FeS_2 aggregation and framboidal pyrite (top left corner)

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Chapter Four: Using reactive transport modeling to predict trace metal removal products in vertical flow bioreactors

This chapter has been formatted and submitted to *Aquatic Geochemistry*.

Abstract

In addition to being used for alkalinity generation, vertical flow bioreactors (VFBR) are often used to remove trace metals as insoluble sulfides via bacterial sulfate reduction (BSR). However, trace metals may be retained in VFBR via several other mechanisms as well, including: adsorption, precipitation as carbonates, complexation with organic matter, and oxide precipitation. Analyses of substrates is the preferred method for determining the fate of trace metals in VFBR, but the work is cost- and labor-intensive. The ability to use reactive transport models to predict VFBR effluent quality and products of trace metal removal would be beneficial. Sequential extraction procedures were performed on substrates from two experimental VFBR and two field-scale VFBR. The sequential extraction procedure included the following fractions: exchangeable, carbonate, labile organic, oxide, refractory organic/sulfide, and residual. Influent water quality data from the experimental VFBR were used to construct and calibrate a reactive transport model in PHREEQC, which was then validated with data from the field VFBR. Overall, geochemical modeling using PHREEQC provided fair predictions of VFBR effluent quality. Model predictions of sulfide, carbonate, and oxide formation were upheld by results of the sequential extraction procedures. Conversely, trace metal removal through complexation with labile organic matter was not well modeled, with concentrations generally being underpredicted.

Manganese removal was not well modeled in general and adjustment of parameters associated with modeling complexation with organic matter may be necessary when predicting VFBR performance.

Introduction

Drainage from abandoned mines containing elevated concentrations of Fe, trace metals, and sulfate continue to pose substantial ecological and human health risks in the US (USEPA 2004, Limerick et al. 2005). These commonly acidic mine drainages may also contain elevated base cation (Na^+, Ca^{2+}, Mg^{2+}) concentrations as a result of secondary reactions and ion exchange processes (Capo et al. 2001, Younger et al. 2002, Watzlaf et al. 2004). Passive treatment systems (PTS) are becoming a more common treatment alternative. These systems are generally composed of a combination of unit processes, including anoxic limestone drains, vertical flow bioreactors (VFBR), and iron oxidation and settling ponds (Younger et al. 2002, Ziemkiewicz et al. 2003, Watzlaf et al. 2004).

Although originally intended as a means to produce anoxic and reducing conditions conducive to generation of bicarbonate alkalinity through bacterial sulfate reduction (BSR) and limestone dissolution (Kepler and McCleary 1994), VFBR are also often used to remove trace metals and sulfate from mine drainage. In addition to removal as insoluble sulfides through BSR, trace metals may also be removed via sorption to organic matter and precipitation as hydroxides and carbonates (Dvorak et al. 1992, Machemer and Wildeman 1992, Webb et al. 1998, Neculita et al. 2008). Although large amounts of sulfate may also be removed through sorption and BSR, efficiency of VFBR in this regard is highly dependable upon substrate composition, hydraulic retention time, and water quality (Dvorak et al. 1992, Cocos et al. 2002, Willow and Cohen 2003, Neculita et al. 2007, Pinto et al. 2015).

The stability and bioavailability of trace metals in VFBR depends upon their removal products, making confirmation of those products very appealing. Laboratory methods, such as sequential extractions and acid-volatile sulfide/simultaneously extracted metals analyses, are often used as a means to identify trace metal removal products. These removal products are generally placed into five increasingly stable categories of species: i) adsorptive and exchangeable, ii) bound to carbonates, iii) bound to Fe/Mn oxides, iv) bound to organic matter and sulfides, and v) bound to primary and secondary minerals (Tessier et al. 1979, Filgueiras et al. 2002, Gleyzes et al. 2002, Jong and Parry 2004).

Although collection and analyses of substrates is the optimal method of determining the fate of trace metals in VFBR, the work is cost- and labor-intensive. The ability to make sound predictions regarding trace metal removal processes in VFBR is beneficial. Geochemical modeling can be an important tool in testing conceptual models for mine drainage treatment prior to implementation (Nordstrom, 2000, Amos et al. 2004, Zagury et al. 2006, Bethke 2008). Reactive transport models may be used to predict not only changes in water quality through a system, but also whether or not processes which lead to the most stable trace metal removal products are occurring (Nordstrom 2000, Amos et al. 2004, Hemsi et al. 2005, Bethke 2008).

A column study was conducted to evaluate the impacts of ionic strength on trace metal removal in VFBR. Two sets of columns utilizing spent mushroom compost as the reactive organic media were fed simulated mine drainages containing elevated concentrations of trace metals at either 10^{-1} M or 10^{-3} M ionic strength for approximately one year. Effluent samples were collected every two weeks and substrate samples were collected at the end of the study. Quantitative simulations of the processes occurring in the column VFBR were conducted using data collected during the study and the geochemical modeling software PHREEQC (Parkhurst and Appelo 1999).

In contrast to many other geochemical modeling programs, PHREEQC may be used with multiple thermodynamic databases and allows modeling of non-equilibrium reactions such as mineral dissolution and precipitation and microbial reactions (Merkel and Planer-Friedrich 2008, Charlton and Parkhurst 2011). This study was conducted to test the VFBR conceptual model based upon data collected during the column study and verification with data collected from two different field sites. An additional goal of this study was to evaluate the ability to predict trace metal removal products as quantified via operationally defined sequential extraction procedures.

Conceptual model

A conceptual model was developed incorporating: anaerobic degradation of spent mushroom compost to simple short-chain carbon molecules, sulfate reduction through oxidation of the short-chain carbon molecules, precipitation of trace metal sulfides, sorption and complexation of trace metals to organic matter, and precipitation of carbonate minerals. Within VFBR, water is forced vertically downward through an organic substrate, creating the anoxic and reducing conditions necessary for BSR. The bacterially mediated reduction of sulfate via oxidation of organic matter (generically represented CH_2O) occurs (Equation 4.1.)

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^{-} + H_2S \tag{4.1}$$

The rate of the reaction in Equation 1 is highly dependent upon the availability of simple, short-chain organic carbon, provided either directly from the substrate or, more commonly, as a result of anaerobic degradation of more complex organic carbon molecules by cellulolytic and fermentative bacteria (Waybrant et al. 1998, Drury 2000, Gibert et al. 2004, Hemsi et al. 2005, Zagury et al. 2006, Choudhary and Sheoran 2011). Activity of sulfate reducing bacteria (SRB) and rate of sulfate reduction are also dependent upon initial sulfate concentrations, with an increase in reaction rates generally corresponding to an increase in sulfate concentration (Chang et al. 2000, Moosa et al. 2002). Some studies have indicated that the rate of decomposition of organic matter does not change significantly over the course of relatively short-term experiments (Waybrant et al. 1998, Amos et al. 2004). As a result, it is possible to combine the rates of organic matter decomposition and sulfate reduction into the reaction and rate expression in Equations 4.2 and 4.3, respectively (Amos et al. 2004).

$$CH_2O + 0.5SO_4^{2-} \rightarrow CO_3^{2-} + 0.5HS^{-} + 1.5H^{+} \tag{4.2}$$

$$\mathrm{R}_{\mathrm{CH_2O-SO_4}} = -\mathrm{k}_{\mathrm{CH_2O-SO_4}} \left[\frac{[\mathrm{SO_4}]}{\mathrm{K_s}+[\mathrm{SO_4}]}\right] \tag{4.3}$$

where $k_{CH_2O-SO_4}$is an effective rate constant (mol/L·s) and K_s is the half saturation constant. Boudreau and Westrich (1984) determined that K_s is equal to 1.62 mmol/L in marine sediments, but that value may be considerably lower in fresh water environments (Roden and Tuttle 1993, Amos et al. 2004). The soluble sulfides produced in Equation 4.1 will then react with divalent cationic metals and bicarbonate to form sparingly soluble metal sulfide precipitates, shown in Equation 4.4.

$$\mathrm{H_2S + M^{2+} + HCO_3^- \rightarrow MS\downarrow + 2H_2O + 2CO_2} \tag{4.4}$$

Although it is possible for some trace metals to coprecipitate with or adsorb to sulfide precipitates, this mechanism is not included in this study.

Another important method of trace metal removal in VFBR, particularly at the beginning of operation, is adsorption to or complexation with organic matter in the substrates, primarily with the humic and fulvic materials (Machemer and Wildeman 1992, Tipping and Hurley 1992, Tipping 1998, Weng et al. 2002, Dudal and Gerard 2004). Humic and fulvic acids are high molecular weight macromolecules that may make up to 70% of dissolved organic matter (Masion et al. 2000, Weng et al. 2002, Appelo and Postma 2005). Tipping and Hurley (1992) developed a model, the Windermere humic acid model (WHAM), to account for complexing of trace metals by humic acid. Equilibrium complexation constants are applied to eight monodentate sites, representing the carboxylic and phenolic binding sites in humic acid, and twelve bidentate sites, which account for the proximity between some binding sites that exist in humic acid (Tipping and Hurley 1992, Tipping 1998, Lofts and Tipping 2000, Appelo and Postma 2005, Parkhurst and Appelo 2013).

Precipitation of carbonate minerals occurs in a similar manner to sulfide precipitation. Carbonate liberated through the dissolution of limestone or produced as a result of BSR may react with trace metals in solution and precipitate (Younger et al. 2002, Watzlaf et al. 2004). The reaction is shown in Equation 4.5.

$$M^{2+} + HCO_3^{-} \rightarrow MCO_3 + H^{+} \qquad (4.5)$$

It is also possible to circumvent the dissolution of limestone or production of bicarbonate and form carbonate minerals through direct surface reactions on limestone (Dvorak et al. 1992), as illustrated in Equation 4.6.

$$M^{2+} + CaCO_3 \rightarrow MCO_3 + Ca^{2+} \qquad (4.6)$$

Model Development

The model constructed in PHREEQC was calibrated using data from a paired comparison column study conducted in the laboratory. Each column measured 30 cm long and 25 cm in diameter. They were filled with a 2:1 ratio of spent mushroom compost and inert river rock to provide substrate stability. Six columns were divided into two sets, one receiving “low” ionic strength (10^{-3} M) and the other receiving “high” ionic strength (10^{-1} M) simulated mine drainage, LI and HI, respectively. Influent water quality characteristics are provided in Table 4.1. The study was conducted for one year, with water quality samples collected approximately every two weeks and substrate samples collected at the end. Each column was modeled in 30, 1-cm control lengths, with simulations allowed to run for the approximate hydraulic residence time of 72 hours.

In addition to the bench-scale VFBR data used to calibrate the models, data from two separate field VFBR were used to verify the applicability of the model to field conditions. The first field VFBR, MPRTS, treats net-alkaline ferruginous mine drainage that has been pre-treated via oxidation, hydrolysis, and precipitation of Fe. This process removes the vast majority of Fe in the mine drainage, as well much of the trace metals. What remains in solution by the time the mine drainage reaches the VFBR is represented by the summary data in Table 4.1. This system was modeled in 40, 1-m control volumes and simulations were run for the estimated hydraulic residence time of 78 hours. The second field VFBR, CC, treats net-acidic ferruginous mine drainage that has not been subjected to any sort of pretreatment. This VFBR is similar in size and construction to MRPTS, but treats substantially different quality water. Summary data provided in Table 4.1 shows this system has a much higher Fe concentration than the other three systems evaluated. This VFBR was also modeled in 40, 1-m control volumes, but simulations were run for an estimated hydraulic residence time of 67 hours.

Table 4.1 Mean influent concentrations for geochemical modeling simulations (blanks indicate data not available)

	LI (n = 8)	**HI (n = 8)**	**MRPTS (n = 66)**	**CC (n = 133)**
T (°C)	21	21	19	19
pH (s.u.)	6.3	6.0	6.4	3.3
pe	7.0	6.5	6.4	4.2
DO (mg/L)	8.1	8.3	8.5	11.6
Alkalinity (mg/L as $CaCO_3$)	5.7	3.2	145	5.38
Sulfate (mg/L)	104	1830	2250	465
Calcium (mg/L)	39	260	700	-
Cadmium (mg/L)	0.48	0.48	0.002	0.002
Cobalt (mg/L)	-	-	0.05	-
Iron (mg/L)	-	-	0.31	76
Manganese (mg/L)	0.49	0.46	1.4	2.7
Sodium (mg/L)	16	707	102	10
Nickel (mg/L)	0.48	0.45	0.76	-
Lead (mg/L)	0.44	0.58	0.03	0.002
Zinc (mg/L)	0.46	0.49	4.8	0.47

Organic and inorganic carbon analyses and sequential extractions were carried out on substrate samples from each VFBR. Carbon data were used to estimate concentrations of humic material and $CaCO_3$ in each system. Alkalinity and pH data were used to estimate the CO_2 concentrations in each solution. A volume-adjusted sulfate removal rate was calculated for each VFBR and used to calculate $k_{CH_2O-SO_4}$ for use in the sulfate reduction rate expression. Parameters used in the model are listed in Table 4.2. Model code and parameters required to model trace metal sorption to humic materials were obtained from the PHREEQC version 3 guide (Parkhurst and Appelo 2013), Tipping and Hurley (1992), and Tipping (1998). All simulations were run using the llnl.dat geochemical database included with PHREEQC.

Table 4.2 Calculated and assumed parameters used in the model

	LI	HI	MRPTS	CC
Humic material (g/L)	0.61	0.60	0.80	0.10
$CaCO_3$ (moles)	0.001	0.001	0.15	0.20
CO_2 (atm)	0.009	0.008	0.035	0.201
$k_{CH_2O-SO_4}$ (mol/L·s)	9 x 10^{-10}	2.4 x 10^{-9}	4.9 x 10^{-9}	3.5 x 10^{-9}

Results and Discussion

Comparison of LI and HI effluents to LI and HI model simulations (Table 4.3) showed that in most cases it was possible to predict VFBR effluent quality reasonably well with PHREEQC. However, notable differences between the measured and modeled concentrations occurred in several cases. Sulfate concentrations were overpredicted in the HI and CC systems and underpredicted in MRPTS. In the case of HI, this is likely due to artificially decreased concentrations in the column effluent as a result of sorption to organic matter at the beginning of the study. Model simulations may actually be more accurate in the long term. The model had a tendency to overpredict Cd concentrations, but underpredict Mn concentrations. Predictions of Fe and Zn concentrations were also inconsistent in the field VFBR.

Table 4.3 Comparison of measured effluent water quality data and simulation results (range = 1st-3rd quartiles)

	LI		**HI**		**MRPTS**		**CC**	
	Measured range	**Model**	**Measured range**	**Model**	**Measured range**	**Model**	**Measured range**	**Model**
pH (s.u.)	7.25 - 7.48	7.96	7.34 – 7.85	8.63	6.68 – 6.79	6.67	6.10-6.38	7.13
Alkalinity (mg/L as $CaCO_3$)	127 – 232	61	175 – 336	359	139 – 171	239	210-380	571
Sulfate (mg/L)	54.6 – 66.3	51	1390 - 1540	1780	2030 – 2100	1380	25.5-183	378
Cd (μg/L)	27 – 31	96	3.9 – 7.0	88	1.0 – 1.0	0.69	0.19 – 0.20	42
Co (μg/L)	-	-	-	-	2.0 – 22	34	-	-
Fe (μg/L)	-	-	-	-	490 – 2120	103	2330 – 12300	20300
Mn (μg/L)	192 - 227	78	377 – 455	27	1350 – 2680	545	1900 – 4050	1910
Ni (μg/L)	33 – 40	68	43 – 58	24	94 – 286	328	-	-
Pb (μg/L)	33 – 48	37	46 – 55	18	-	14	2.0 – 2.0	10
Zn (μg/L)	37 - 47	74	15 - 20	30	46 - 1150	2058	10 – 34.5	344

Results of sequential extractions on substrates from all four VFBR are presented in Table 4.4. The carbonate, labile organic, oxide, and refractory organic/sulfide fractions may all be represented in the modeled simulations. The labile organic fraction played an important role in trace metal removal, particularly Fe and Zn, and was represented in the model by the WHAM component. Removal via the carbonate, oxide, and refractory organic/sulfide fractions were represented by the saturation indices of minerals provided in the LLNL database. Modeling the exchangeable fraction requires experimental determination of the cation exchange capacity (CEC) in the substrate being modeled. However, the vast majority of the CEC in organic substrates used in VFBR is contributed by humic materials (Bradl 2004). Therefore, any trace metals retained in the exchangeable fraction will be represented in the results of the WHAM component of the model.

Table 4.4 Summary results of sequential extractions on substrate samples from four VFBR (median ± interquartile range)

	LI	**HI**	**MRPTS**	**CC**
Exchangeable				
Cd (mg/kg)	1.3 ± 1.2	1.2 ± 1.1	<0.01	0.12 ± 0.41
Co (mg/kg)	-	-	0.019 ± 0.19	-
Fe (mg/kg)	-	-	<0.01	390 ± 324
Mn (mg/kg)	150 ± 62	130 ±15	60 ± 26	94 ± 61
Ni (mg/kg)	0.73 ± 0.65	0.73 ± 0.91	5.0 ± 3.6	-
Pb (mg/kg)	1.4 ± 1.2	0.23 ± 0.43	<0.35	1.3 ± 0.62
Zn (mg/kg)	1.2 ± 0.79	2.2 ± 1.2	2.5 ± 1.1	1.9 ± 4.3
Carbonate				
Cd (mg/kg)	5.8 ± 6.6	11 ± 11	<0.03	0.11 ± 0.12
Co (mg/kg)	-	-	1.1 ± 1.0	-
Fe (mg/kg)	-	-	110 ± 89	197 ± 278
Mn (mg/kg)	220 ± 35	245 ± 45	92 ± 26	59 ± 32
Ni (mg/kg)	3.5 ± 6.6	6.6 ± 9.6	48 ± 32	-
Pb (mg/kg)	7.9 ± 12	3.9 ± 6.2	<0.97	7.5 ± 2.1
Zn (mg/kg)	27 ± 12	34 ± 18	130 ± 57	19 ± 14
Labile Organic				
Cd (mg/kg)	18 ± 18	43 ± 35	0.019 ± 0.023	1.0 ± 0.86
Co (mg/kg)	-	-	4.4 ± 1.7	-
Fe (mg/kg)	-	-	940 ± 460	393 ± 311
Mn (mg/kg)	53 ± 5.8	65 ± 15	19 ± 4.9	15 ± 8.0
Ni (mg/kg)	15 ± 21	32 ± 36	110 ± 33	-
Pb (mg/kg)	23 ± 22	22 ± 20	1.4 ± 0.67	1.8 ± 0.89
Zn (mg/kg)	135 ± 34	184 ± 53	547 ± 108	19 ± 4.9
Oxide				
Cd (mg/kg)	5.3 ± 5.6	4.0 ± 2.9	0.041 ± 0.024	0.16 ± 0.07
Co (mg/kg)	-	-	1.3 ± 0.93	-
Fe (mg/kg)	-	-	407 ± 196	342 ± 62
Mn (mg/kg)	8.2 ± 2.1	11 ± 1.7	26 ± 9.5	17 ± 11
Ni (mg/kg)	1.9 ± 4.1	4.2 ± 6.2	46 ± 17	-
Pb (mg/kg)	5.4 ± 6.7	3.0 ± 2.8	0.58 ± 0.086	0.61 ± 0.23
Zn (mg/kg)	39 ± 12	46 ± 17	358 ± 156	42 ± 14
Refractory organic/sulfide				
Cd (mg/kg)	110 ± 130	200 ± 220	0.83 ± 0.19	2.5 ± 1.0
Co (mg/kg)	-	-	60 ± 39	-
Fe (mg/kg)	-	-	5600 ± 1360	6020 ± 2660
Mn (mg/kg)	44 ± 4.9	46 ± 5.1	62 ± 16	53 ± 24
Ni (mg/kg)	110 ± 140	280 ± 260	1140 ± 495	-
Pb (mg/kg)	120 ± 140	150 ± 140	8.2 ± 1.7	12 ± 2.5
Zn (mg/kg)	450 ± 140	640 ± 270	16100 ± 6130	149 ± 44
Residual				
Cd (mg/kg)	0.051 ± 0.072	0.10 ± 0.11	0.030 ± 0.014	0.083 ± 0.046
Co (mg/kg)	-	-	0.69 ± 0.65	-
Fe (mg/kg)	-	-	320 ± 126	1460 ± 1210
Mn (mg/kg)	2.2 ± 0.27	2.4 ± 0.43	2.1 ± 0.61	7.8 ± 6.7
Ni (mg/kg)	0.26 ± 0.062	0.10 ± 0.19	12 ± 26	-
Pb (mg/kg)	0.76 ± 0.40	0.72 ± 0.59	0.34 ± 0.12	1.0 ± 0.43
Zn (mg/kg)	1.7 ± 0.28	2.2 ± 0.35	98 ± 174	10 ± 9.0

All four VFBR were supersaturated with respect to CdS, which is in agreement with results of the sequential extractions. However, a large percentage of Cd was also found in the labile organic fractions of LI, HI, and CC. The fact that effluent concentrations in these systems were overpredicted in the model indicates the possibility that the fraction bound to humic substances was not estimated well. Substantial percentages of Cd were found in the carbonate fraction in LI, HI, and CC VFBR, in agreement with supersaturation with respect to otavite ($CdCO_3$) predicted by the model.

Removal of Fe was overpredicted in the MRPTS simulation and underpredicted in the CC simulation. The largest percentage of Fe in both VFBR was found in the refractory organic/sulfide fraction, in agreement with the supersaturated conditions with respect to pyrite predicted by the model. Siderite ($FeCO_3$) precipitation was not predicted in MRPTS, although a sizable concentration of Fe was found in the carbonate fraction of the sequential extraction. This discrepancy may account for the difference in measured and modeled effluent concentrations. On the other hand, siderite precipitation was predicted in CC and a good deal of Fe was found in the carbonate fraction of the sequential extraction. Much more Fe was found in the exchangeable fraction in CC than in the other three VFBR. This removal product was not accounted for in the model and was likely the cause for overprediction of effluent Fe concentration.

Effluent Zn concentrations were greatly overpredicted in MRPTS and CC simulations. Sphalerite (ZnS) and wurtzite ((Zn,Fe)S) were predicted to precipitate in both VFBR, which was confirmed by the large percentage of Zn found in the refractory organic/sulfide fraction of the sequential extractions performed on those substrates. In addition, comparatively small concentrations of Zn were found in the labile organic fraction of the sequential extraction procedure, indicating that the model was not likely to underpredict complexation with humic materials enough to account for the discrepancy in measured and modeled effluent concentrations. Both MRPTS and CC were undersaturated with respect to smithsonite ($ZnCO_3$), which was corroborated by the small percentages of Zn in the carbonate fraction of the sequential extraction procedure. It is believed the most likely cause for the large disagreement between measured and modeled effluent Zn concentrations in MRPTS and CC was the seasonal variations in removal via BSR. The simulations were run at a relatively cool 19°C, while temperatures in the VFBR can reach as high as 30°. This substantial increase in temperature stimulates greater SRB activity, increasing the rate of removal of Zn through sulfide precipitation. This may result in a decrease in average effluent Zn concentrations, causing disagreement with modeled predictions.

Effluent Mn concentrations were not well modeled in LI, HI, and MRPTS VFBR. Simulated effluent concentrations were much less than measured concentrations. Rhodochrosite ($MnCO_3$) was only predicted to precipitate in CC, but all of the VFBR contained high concentrations of Mn in the carbonate fraction of the sequential extraction procedure. This implies that modeled Mn concentrations should have been greater than measured. In addition, the large percentage of Mn in the exchangeable fraction of the sequential extraction procedure, which was not explicitly included in the model, should also have caused simulated Mn concentrations to be larger than measured. Despite these two conditions, modeled concentrations were much lower than measured concentrations. Given the high solubility of Mn-sulfide minerals and the fact that none were predicted to precipitate, it must be assumed that the fraction bound to humic materials was greatly overestimated.

Conclusions

Overall, geochemical modeling using PHREEQC provided fair predictions of VFBR effluent quality. In particular, the predictions of precipitation of sulfide minerals in the simulations were upheld by results of sequential extraction procedures. Model predictions of precipitation of carbonates were also well substantiated. In contrast, removal of trace metals through complexation with humic materials did not seem to be well modeled. In instances when a large percentage of a metal was found in the labile organic fraction of the sequential extraction procedures, the model generally overpredicted the VFBR effluent concentration, indicating removal via this mechanism was underpredicted. Manganese removal was not well modeled in general. Only one VFBR (CC) was supersaturated with respect to rhodochrosite and no other Mn minerals were predicted to precipitate. It is believed that the WHAM component of the model overpredicted the concentration of Mn associated with humic materials, artificially decreasing the predicted effluent concentrations. Manipulation of the equilibrium binding constants associated with the WHAM modeled may be required to generate more accurately simulated effluent conditions.

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