Project Report

Haystack Mine Water:

Bench-Scale Testing of Anaerobic Bioremediation Using Local Substrates

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May 2, 2016

1 Project Description and Objectives

1.1 Problem Definition/Background

Mine Impacted Water (MIW), generated in active and abandoned mine sites, contains elevated levels of toxic metals and sulfates that can have an adverse impact on the environment. The major pathway involved in the generation of MIW is weathering of pyritic mineral ores, where sulfur is oxidized to sulfate followed by the release of metal ions. The low pH of the MIW generally leads to higher metal concentrations that are active and labile in the aqueous phase. Increased awareness of metal toxicity and the extent of water pollution stimulated regulators to investigate new alternatives for metal removal treatments from MIW.

In recent years, new MIW treatments focused on passive remediation techniques because of its potential for low cost, low maintenance, and reduced amounts of hazardous waste requiring disposal. Passive remediation using sulfate reducing bioreactors (SRBRs) is a promising approach to MIW treatment. SRBRs use heterotrophic or autotrophic anaerobes called Sulfate Reducing Bacteria (SRB), and offer the advantages of low operational costs and high metal removal even with acidic influent and in seasonally unaccessible sites. The main mechanism of the SRBR process is the microbially-mediated reduction of sulfate to sulfide using a carbon source as the electron donor. Upon sulfate reduction, metal precipitation and co-precipitation occurs via the formation of metallic sulfides, hydroxides, and carbonates.

Many times, the consortia existing in the treated water is enough to generate the required microbial populations to treat the water, but in several cases, bioaugmentation is needed. Common external inoculum sources can be sediments, manure, or biomass from other operational SRBRs (bioaugmentation). In this study, the two selected substrates contained local materials only: 1) wood chips, hay, horse manure, and limestone for the ligneous substrate, and 2) Mackay Gulch soil, quartz sand, horse manure, and limestone for the soil-based substrate. The Mackay Gulch soil was collected from overburden material excavated at a mine tailings repository in Montana and was classified as sandy soil according to its particle size.

The Haystack Creek Mine is an abandoned mine in Cascade County, MT, which is part of the Carpenter Snow Creek Mining District Superfund Site. Previous reports have identified arsenic, cadmium, copper, iron, manganese, nickel and zinc in surface water samples near to the mine in levels exceeding the background concentrations found in the area creeks. The Haystack Creek MIW was characterized for elemental content (Table 1.1 shows the results), and zinc (Zn) was selected as the main target contaminant to be removed and used to evaluate the efficiency of the substrates, but other metals (e.g., Al, Cu, Fe, Mn, Cd are also reported in this study). The anions in the Haystack Creek water were dominated by sulfate (Table 1.2), an anion necessary for SRB activity, while the pH was acidic.

Table 1.1 Elemental Composition in Water Collected from the Haystack Creek Mine Site

Haystack Creek Mine Water			
Analyte	**Unit**	**Total Elements**	**Dissolved Elements**
Aluminum	(mg/L)	1.50	1.21
Antimony	(mg/L)	0.06	<0.008
Arsenic	(mg/L)	0.05	<0.007
Barium	(mg/L)	0.08	0.22
Cadmium	(mg/L)	0.04	0.03
Calcium	(mg/L)	58.13	0.02
Chromium	(mg/L)	0.06	<0.038
Cobalt	(mg/L)	0.15	0.03
Copper	(mg/L)	0.09	0.03
Iron	(mg/L)	0.59	0.19
Lead	(mg/L)	<0.25285	<0.25286
Magnesium	(mg/L)	6.85	5.60
Manganese	(mg/L)	1.14	1.10
Nickel	(mg/L)	0.08	0.02
Potassium	(mg/L)	2.20	2.15
Selenium	(mg/L)	0.06	<0.015
Silicon	(mg/L)	20.51	20.26
Silver	(mg/L)	<0.0053	<0.0054
Sodium	(mg/L)	10.17	10.44
Strontium	(mg/L)	0.29	0.26
Thallium	(mg/L)	ND	ND
Vanadium	(mg/L)	0.06	<0.026
Zinc	(mg/L)	4.22	4.57

Table 1.2 Anions and pH in the Haystack Creek Mine Water

Haystack Creek Mine Water		
Analyte	**Unit**	**Concentration**
Fluoride	(mg/L)	1.55
Chloride	(mg/L)	0.55
Nitrite	(mg/L)	0.65
Sulfate	(mg/L)	195.64
Bromide	(mg/L)	<0.017
Nitrate	(mg/L)	<0.13
Phosphate	(mg/L)	<0.07
pH	pH Units	3.82

1.2 Project Objectives

The primary objective of this work was to evaluate metal removal from the Haystack Creek MIW under anaerobic conditions using two column bioreactors with different substrates: the first constituted by wood chips, hay, horse manure, and limestone, and the second consisting of Mackay Gulch soil, sand, horse manure, and limestone.

2 Experimental

2.1 Experimental Design

The metal removal evaluation was performed using anaerobic column bioreactors. The two experimental columns received influent water at the same flowrate from the same reservoir, the only difference was the substrates in each column. No control columns or replicates were considered since the main project objective was to test the viability of the local materials as substrate. Approximately 80 L of Haystack Creek MIW was collected and shipped to the EPA/ORD Center Hill Facility in Cincinnati, OH for this study. The MIW and solid substrates were stored inside sealed containers at 4ºC until use in the experiment. The solid substrates were characterized for moisture content, elemental composition, and total organic carbon percentage.

The column mixtures were designed to contain 30 g of total organic carbon in each column, accounting for total organic carbon percentage and moisture content of each component of the substrate mixtures. Column 1, the ligneous substrate column, was loaded with wood chips, hay, and horse manure to make the 30 g of organic carbon and with the addition of 20 g of limestone (see Table 2.1 for details). Column 2, the manure-based substrate column, was loaded with a mixture of the Mackay Gulch soil, horse manure, quartz sand, and was also complemented with 20 g of limestone. The limestone was added to add alkalinity to the substrate aiming to provide a neutral environment for SRB growth. The high purity sand was previously digested to obtain elemental composition and it was determined that here is no need to wash it prior to loading it into Column 2. The sand was premixed with the other substrate materials to ensure substrate porosity because fine grain soils, like the McKay Gulch Soil, tend to 'cake' when wetted, and don't allow water to permeate through the soil easily.

Table 2.1. Columns substrate content. The amounts were established based on total carbon content and moisture content of each material

Ligneous Substrate Content					
Material	**% TOC**	**Required Carbon Mass (g)**	**Mass of the material (g)**	**Moisture (%)**	**Substrate content (%) w/w**
Wood chips	46.66	12.0	25.72	8.62%	**17%**
Hay	26.10	12.0	45.97	51.30%	**30%**
Horse Manure	9.90	6.0	60.61	52.40%	**41%**
Limestone	5.89	1.18	20.00	0.00%	**13%**

Manure-based Substrate Content					
Material	**% TOC**	**Required Carbon Mass (g)**	**Mass of the material (g)**	**Moisture (%)**	**Substrate content (%) w/w**
McKain-Goulch Soil	1.38	0.30	21.67	11.85%	**7%**
Horse Manure	9.90	29.7	300.00	72.69%	**93%**
Limestone	5.89	1.18	20.00	0.00%	**6%**
Sand	0.00	0.0	500.00	0.00%	**-**

The substrate materials did not contain significant amounts of metals according to their elemental composition obtained through acid digestion (Table 2.2).

Table 2.2 Elemental composition of the substrate materials.

Element	Units	Wood Chips	Hay	Horse Manure	McKay Gulch Soil	CSC Limestone
Al	mg/kg	<47.1	57.0	366	27100	697
As	mg/kg	<1.35	<1.35	<1.35	<1.35	2.61
Ba	mg/kg	7.72	17.4	85.3	198	36.8
Ca	mg/kg	673	6100	5760	5470	371000
Cd	mg/kg	0.093	<0.071	0.297	1.24	0.297
Co	mg/kg	<0.21	<0.21	0.316	31.6	1.60
Cr	mg/kg	<7.19	<7.19	<7.19	126	<7.19
Cu	mg/kg	<0.713	5.50	10.3	156	8.77
Fe	mg/kg	<5.66	90.8	587	38200	957
K	mg/kg	311	19400	6220	3660	152
Mg	mg/kg	125	1850	2380	11500	2510
Mn	mg/kg	65.4	32.4	256	458	94.2
Na	mg/kg	20.1	7260	2020	771	161
Ni	mg/kg	<0.434	1.27	3.43	48.7	1.65
P	mg/kg	14.4	2030	4000	826	82.9
Pb	mg/kg	<42.9	<42.9	<42.9	286	52.0
Se	mg/kg	<2.92	<2.92	<2.92	<2.92	<2.92
S	mg/kg	47.1	1450	1860	1380	89.9
Si	mg/kg	<31.8	551	720	389	262
Sr	mg/kg	2.60	31.7	82.1	104	87.0
V	mg/kg	<4.97	<4.97	<4.97	46.5	6.57
Zn	mg/kg	7.27	21.1	58.5	346	40.5

Metal removal efficiency was assessed by comparing the influent/effluent metal concentrations. The MIW was loaded into 11-liter reservoir and placed inside an anaerobic chamber. The water was pumped from the reservoir to fill the columns from bottom to top. The columns were left idle to promote microbial growth for 7 weeks. Following this period, the MIW was pumped through the columns at a rate of ~2 L/week/column. The water exited through a port at the side of the column and was collected in Tedlar bags (See Fig. 1 for details). Samples of influent and effluent water were taken weekly to measure for pH, alkalinity, dissolved elements, dissolved oxygen, and sulfate concentration. During the course of the experiment, oxidation reduction potential (ORP) was monitored in-line and recorded every 15 minutes on a computer, the data was reduced with a daily average and converted to redox potential E_h.

At the conclusion of the 18-week experimental period, the columns were drained of the remaining aqueous solution. The solid residues were collected in three portions (top, middle, bottom) and oven-dried. The dried solids were characterized for elemental composition by acid digestion (EPA Method 3051) and further ICP analysis.

Fig. 1. Picture of the experimental columns at the 16th week of operations: the ORP probes were right above the glass beads and the effluent line was 24 cm above the glass beads level.

2.2 Methods

2.2.1 Solids characterization

The solid substrates were subjected to initial characterization analyses detailed in QAPP L20963-QP-1-3, which included the following parameters:

- Moisture: Moisture content of the solid materials allowed the calculation of the mass of each component to be added into the columns on a dry weight basis.
- Elemental composition: the elemental composition of the substrate materials was determined because these materials may leach some amounts of contaminants that may interfere with the metal removal results.
- Total Organic Carbon (TOC): determined the mass of the substrate materials added into each column to keep the desired organic carbon content.

2.2.2 Aqueous phase weekly analyses

Weekly analyses were performed on the influent and effluent samples of the columns as described in QAPP L20963-QP-1-3:

- pH: pH is a critical factor for the biomass to have a suitable environment to thrive. The desired range would be 5.5-7.5 inside the column and was monitored at the effluent. The influent value was not adjusted and reflected the pH of the collected mine water.
- Alkalinity: Since the influent Haystack Creek mine water was acidic, an alkaline substrate was needed to neutralize that acidity and to generate a neutral environment for SRB growth. The substrate alkalinity was provided by the limestone (calcium carbonate), but microbial activity within the reactor also increases alkalinity, although in lesser amounts than the limestone. The alkalinity was also measured in the effluent.
- Dissolved Metals: This is an important parameter to measure the efficacy of the treatment. The performance of the reactor could be measured by a percentage removal of certain metal(s) of interest by establishing an effluent level that is desirable.
- Sulfate: Since the reactors remove metals by sulfate reduction to sulfates, this parameter is determinant in learning if the SRB are performing well and if the sulfate reduction was the main metal removal mechanism.

3 Results

3.1 Columns

The columns containing the Haystack Creek Mine substrates and MIW sat idle for 7 weeks to promote microbial growth. After that, MIW was pumped through the columns for 18 weeks. Sufficient growth was determined by visual inspection of the organic matter in the columns (the substrate turned black), and gas generation (the H_2S odor was perceived) observed in the Tedlar bags. Each week, the influent and effluent samples were tested for pH, alkalinity, dissolved elements, sulfates, and sulfides.

The porosity of the substrate is important because it allows continuous operation without clogging the reactors. The porosity of the substrate was measured and used to calculate the hydraulic retention time (HRT) of the columns. Operational parameters and the measured porosity of the two experimental columns is reported in Table 3.1.

Table 3.1 Operational variables in the columns: flowrate, total volume, porosity, porous volume, and hydraulic retention time (HRT)

Parameter	Unit	Ligneous Substrate Column	Manure-based Substrate Column
Flowrate	mL/h	12.04	11.95
Total Volume	mL	1158	1158
Average Porosity	%	69.9	38.1
Porous Volume	mL	809	441
HRT	h	67.2	36.9

The HRT used in these columns were reduced from those used in the Big Seven Mine experiments (those were 133 and 118 h) because in previous experiments with different mine water chemistry, we were able to obtain successful sulfate and metal removal with HRT in the range of 48 h.

3.1.1 Flow rate

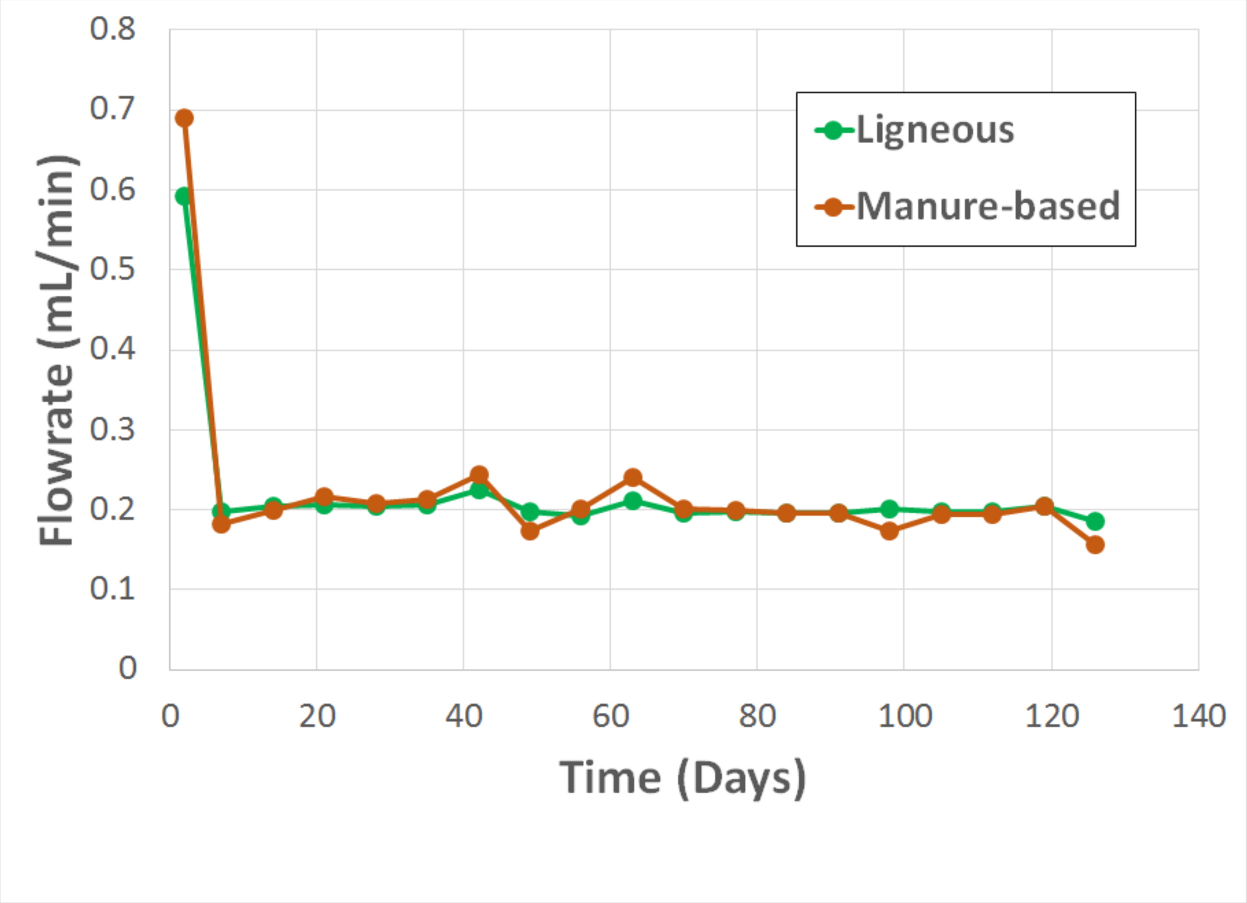


Figure 3.1 Weekly Flowrate Measurement

The flowrate remained constant with an average 0.20 mL/min (12.04 mL/h in the ligneous substrate column and 11.95 mL/h in the manure-based substrate column).

3.1.2 pH Analysis

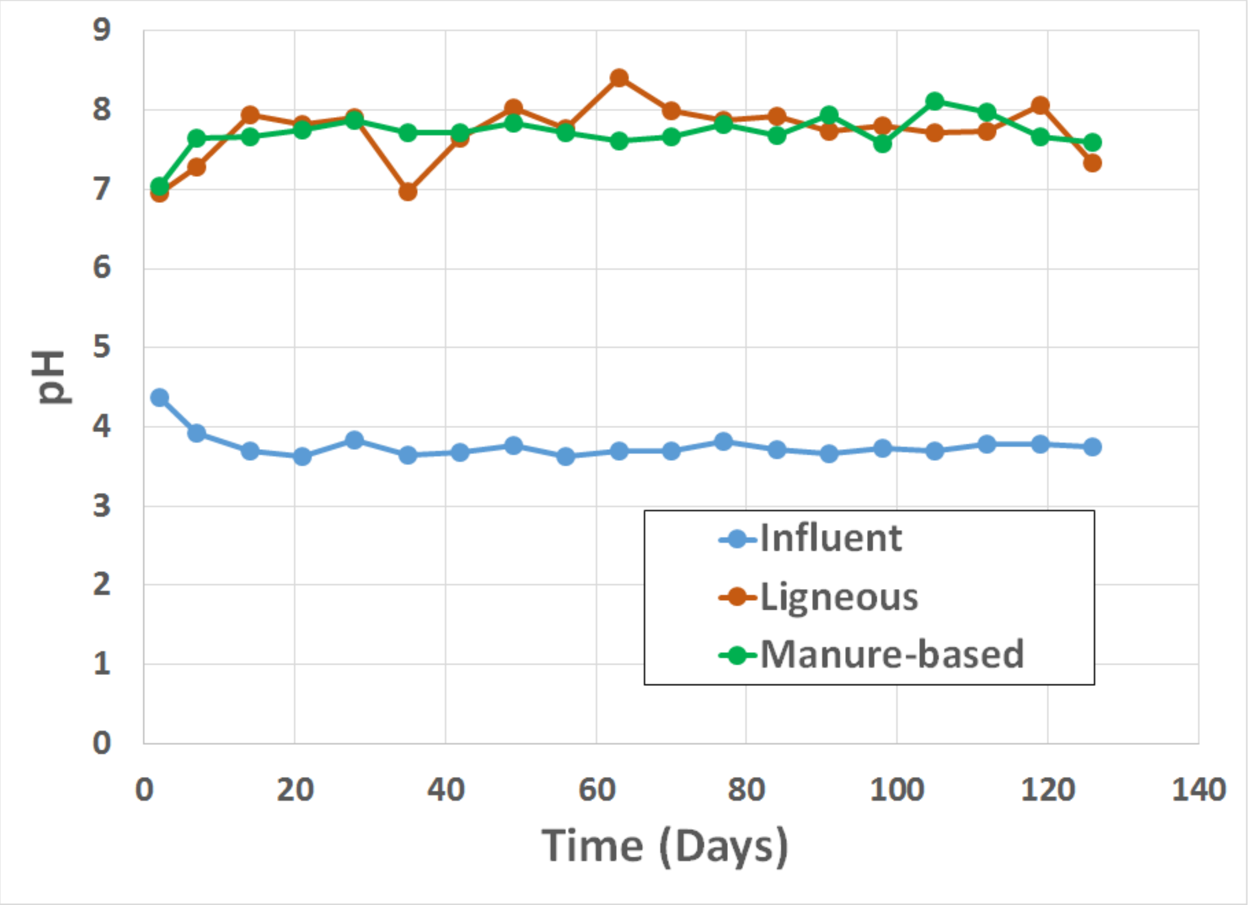
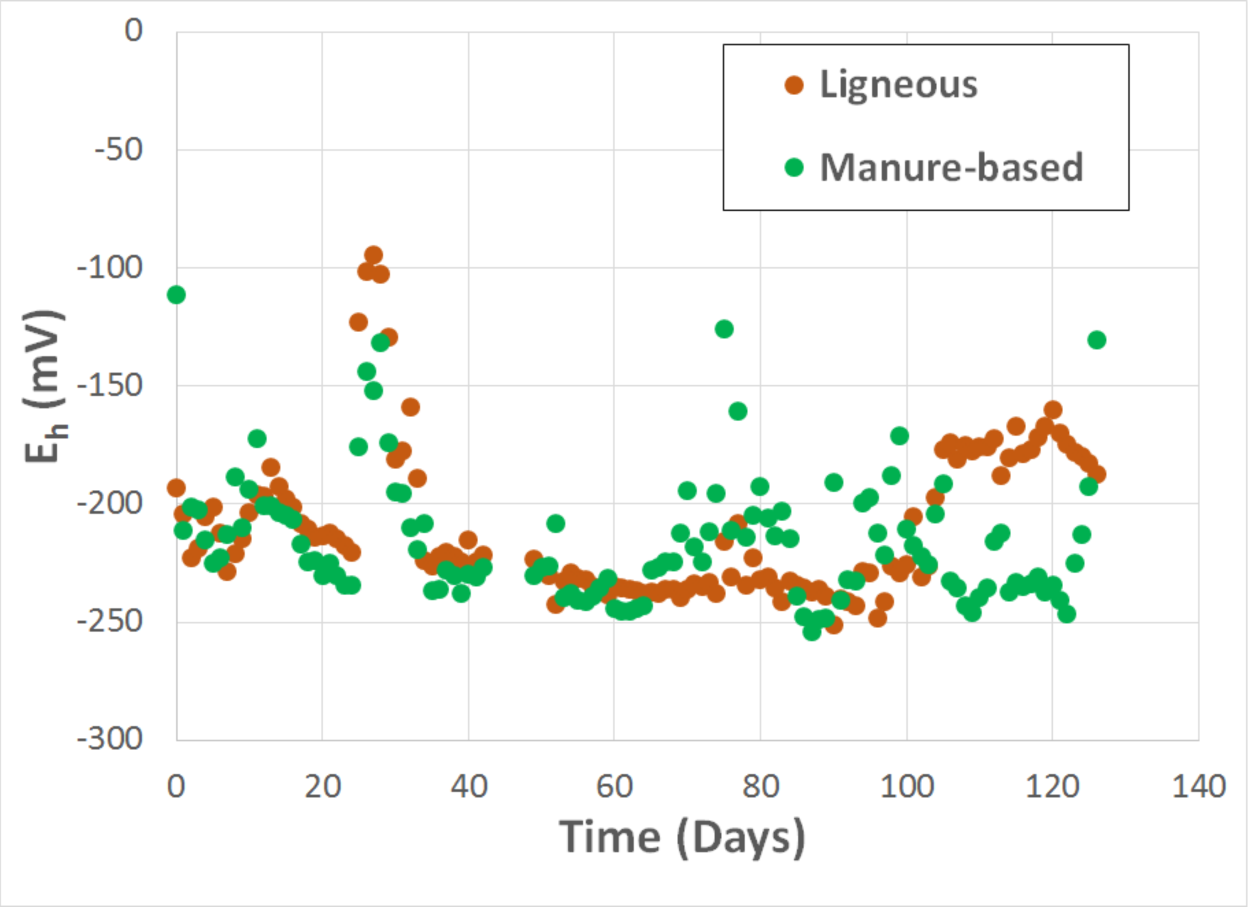


Figure 3.2 Weekly pH Measurement

Over the 18-week experimental period, the Haystack Creek MIW used as influent had small pH variations (average pH was 3.76). The limestone in the substrate generated neutral effluent with an average pH of 7.73 in the ligneous substrate column and 7.72 in the manure-based substrate column.

3.1.3 Redox Potential (E_h)



Redox potential was always in the negative (reducing) during the whole experimental period. This was very important to obtain actual sulfate reduction and is a consequence of the effective neutralization of the influent. There were a few episodes of increased E_h in the columns that reached the -100 mV vicinity, but were always kept in the reducing conditions side at neutral pH. The average E_h in the ligneous substrate column was -210 mV, while the E_h in the manure-based substrate column was -216 mV.

3.1.4 Alkalinity

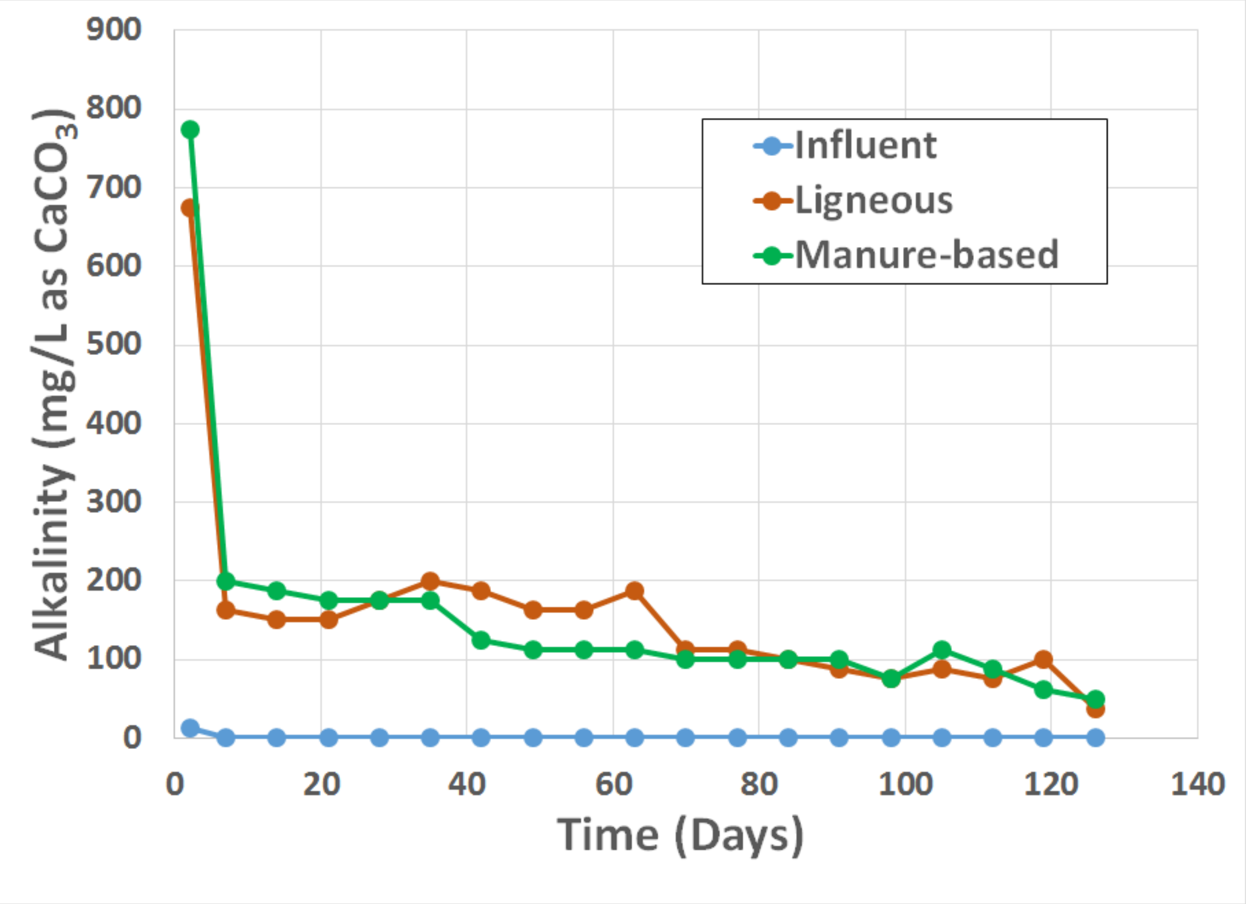


Figure 3.3 Weekly Alkalinity Measurement

Alkalinity had a very high concentration in the first week as a consequence of the stagnant period, and was diluted to <200 mg/L as $CaCO_3$ from then on and was continuously decreasing. At the end of the run, the alkalinity values were -38 mg/L as $CaCO_3$ for the ligneous substrate column and 50 mg/L as $CaCO_3$ for the manure-based substrate column. This decrease in alkalinity could be due to the depletion of the calcium carbonate supplied by the limestone and could be the cause of the observed sulfate breakthrough, which subsequently motivated the metals breakthrough. If the alkalinity depletion continued at the same rate as the rate observed from weeks 2 to 18, it is expected that alkalinity will be completely depleted by the 25th week with the current limestone load (0.4 g of limestone/L of treated water). This load should be increased to at least 0.84 g of limestone/L of treated water to have a one-year alkalinity source with the used particle size of 2 mm.

3.1.5 Sulfates

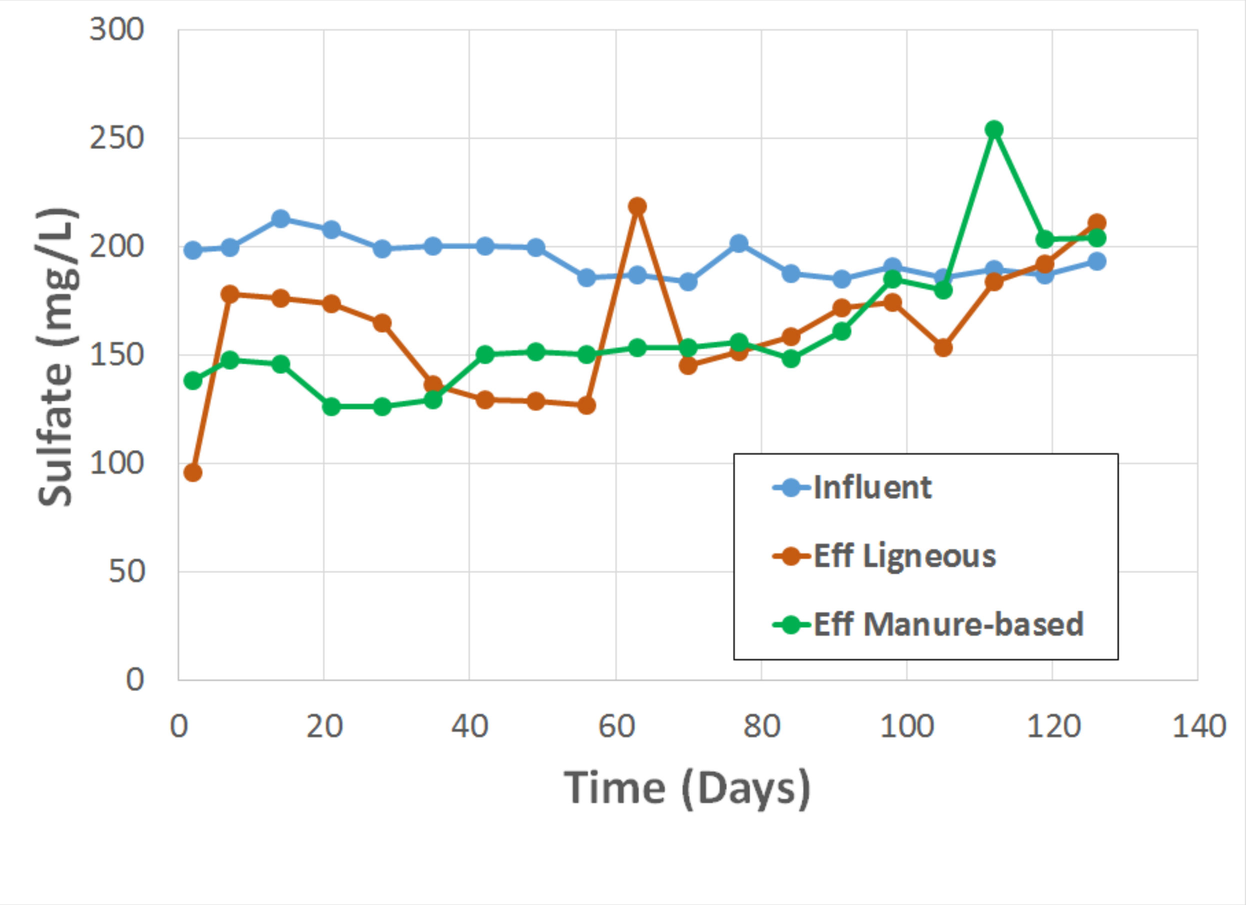


Figure 3.5 Weekly Sulfate Measurement

The ligneous substrate column started with a low sulfate content, but increased after the first week to reach a plateau, then suffered some variation, including an outlier at the 9th week. Nevertheless, an evident breakthrough took place in the last three weeks of operations. The manure-based substrate column started with a steady sulfate concentration near 150 mg/L, but increased from week 13 and experienced breakthrough in the last 3 weeks, in which the effluent sulfate was at higher concentrations than the influent.

3.1.6 Dissolved Metals

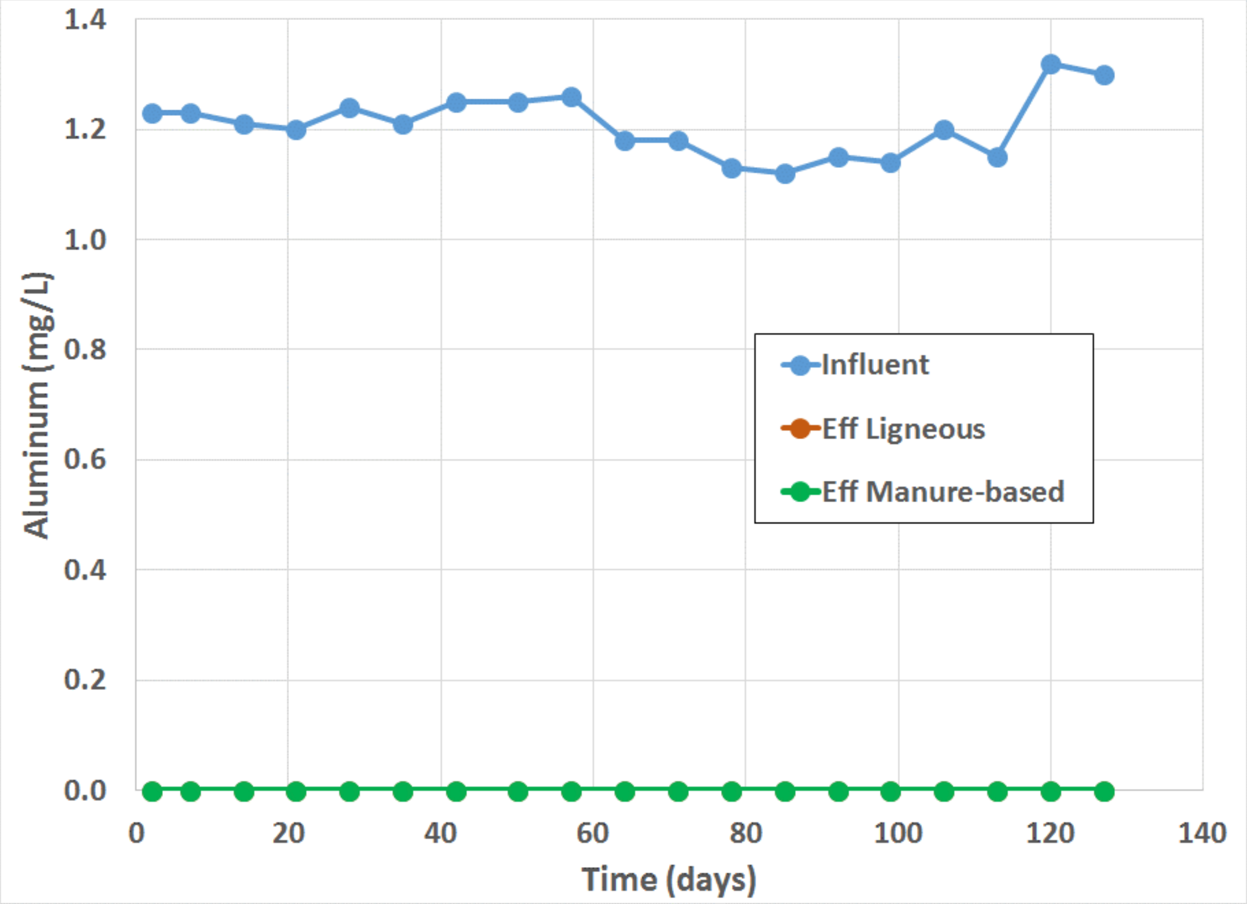


Figure 3.6 Weekly Aluminum Measurement

Aluminum was continuously removed from both columns throughout the experimental period.

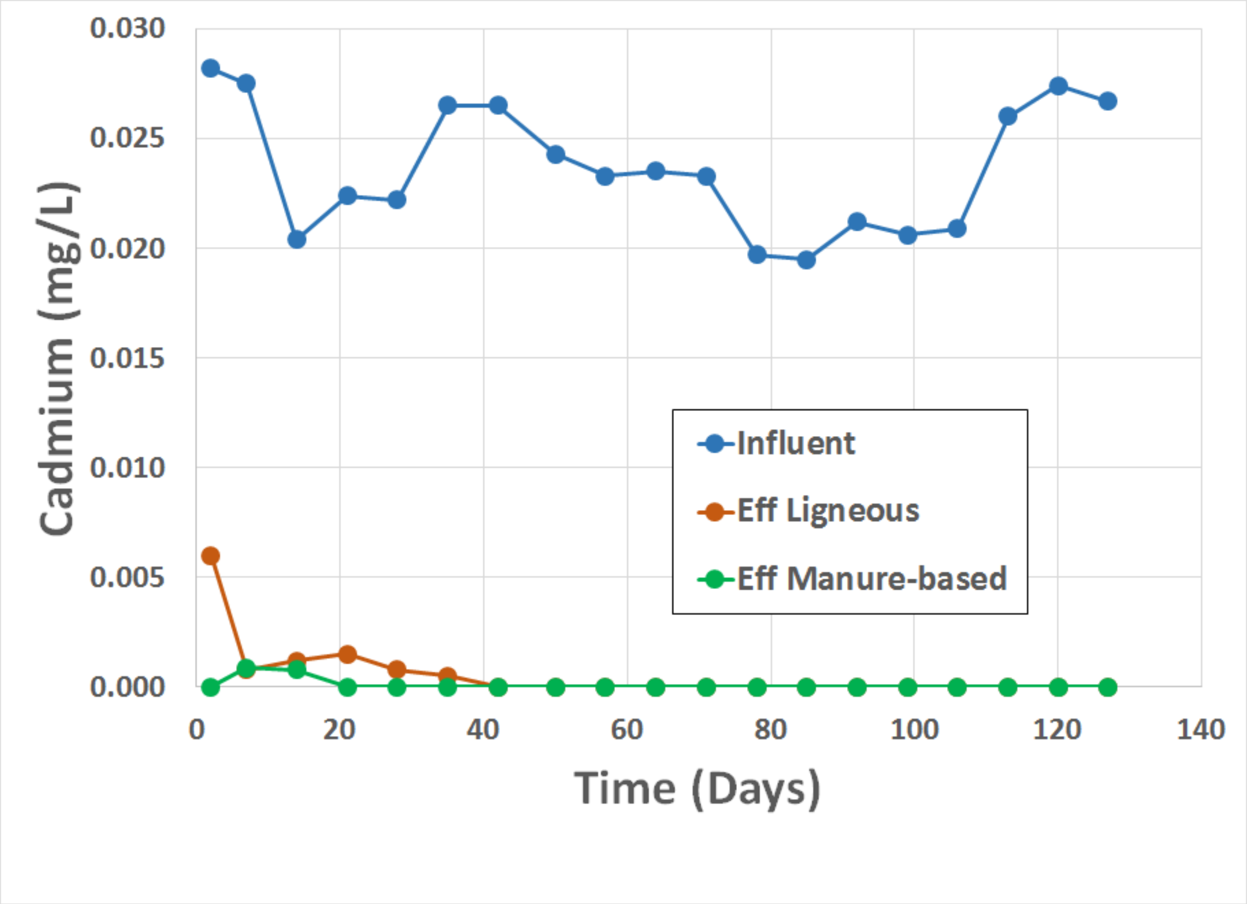


Figure 3.7 Weekly Cadmium Measurement

Cadmium was present in low concentrations in the influent Haystack MIW and was consistently removed in both columns to undetectable concentrations.

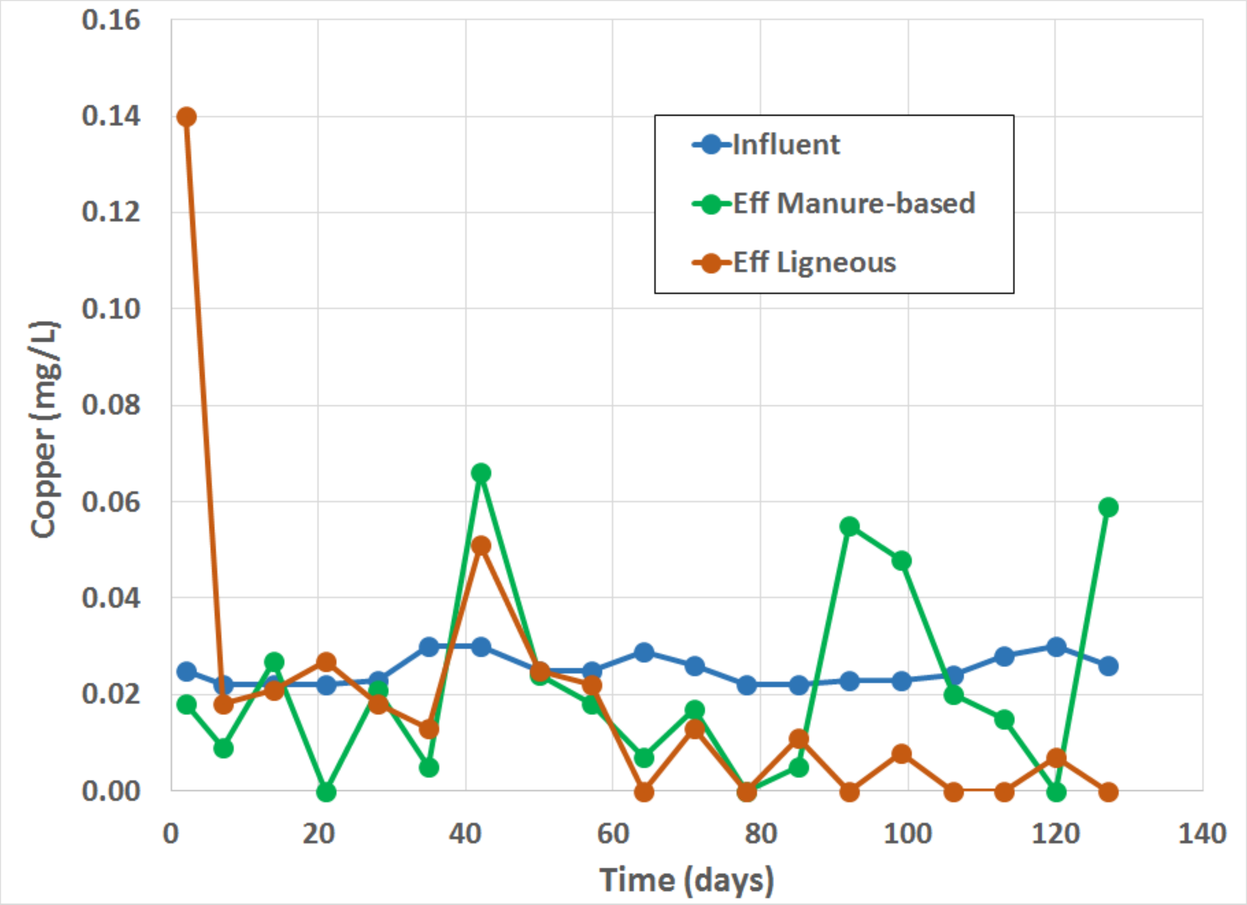


Figure 3.8 Weekly Copper Measurement

Copper was removed in both columns, but had variations in its effluent concentrations. The manure-based substrate columns had higher concentrations in the final weeks of the operational period.

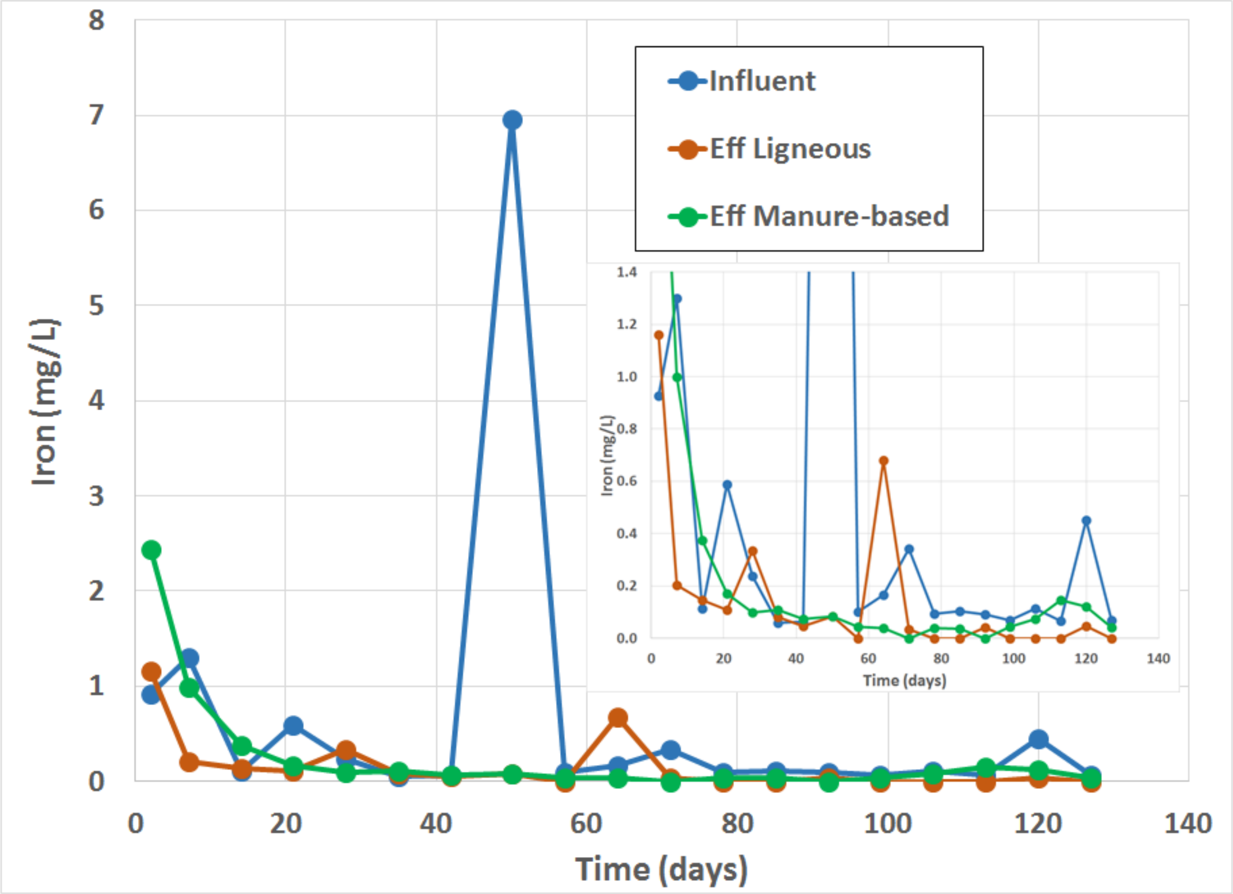


Figure 3.9 Weekly Iron Measurement

Iron had an outlier in the 6th week influent but was removed in both columns. Both columns had small variations in the effluent iron concentration, with the ligneous substrate column being more consistent at the end of the run.

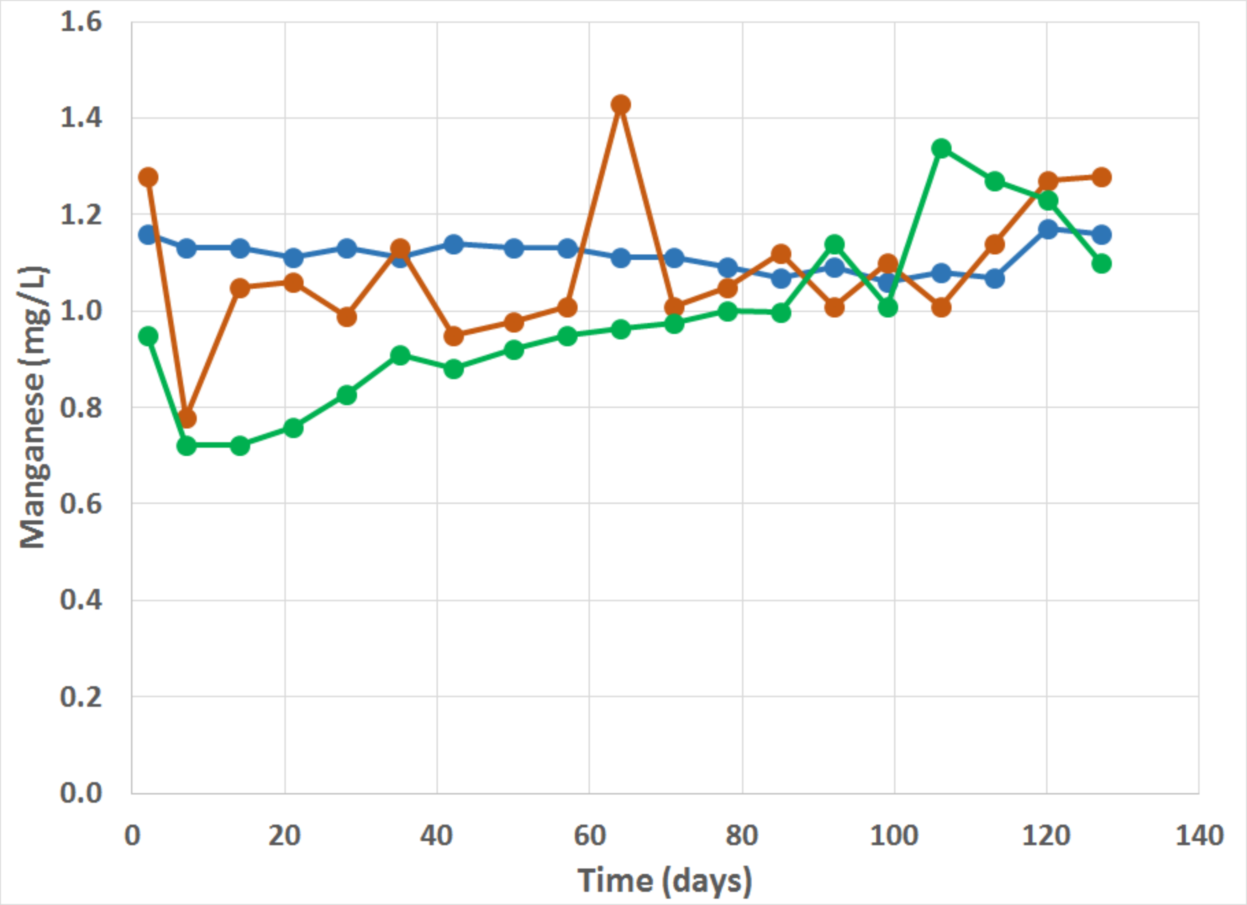


Figure 3.10 Weekly Manganese Measurement

Manganese was present in the Haystack MIW at an average concentration of 1.11 mg/L, and was removed at low amounts in the initial period, but had a breakthrough by the 13th week in both columns.

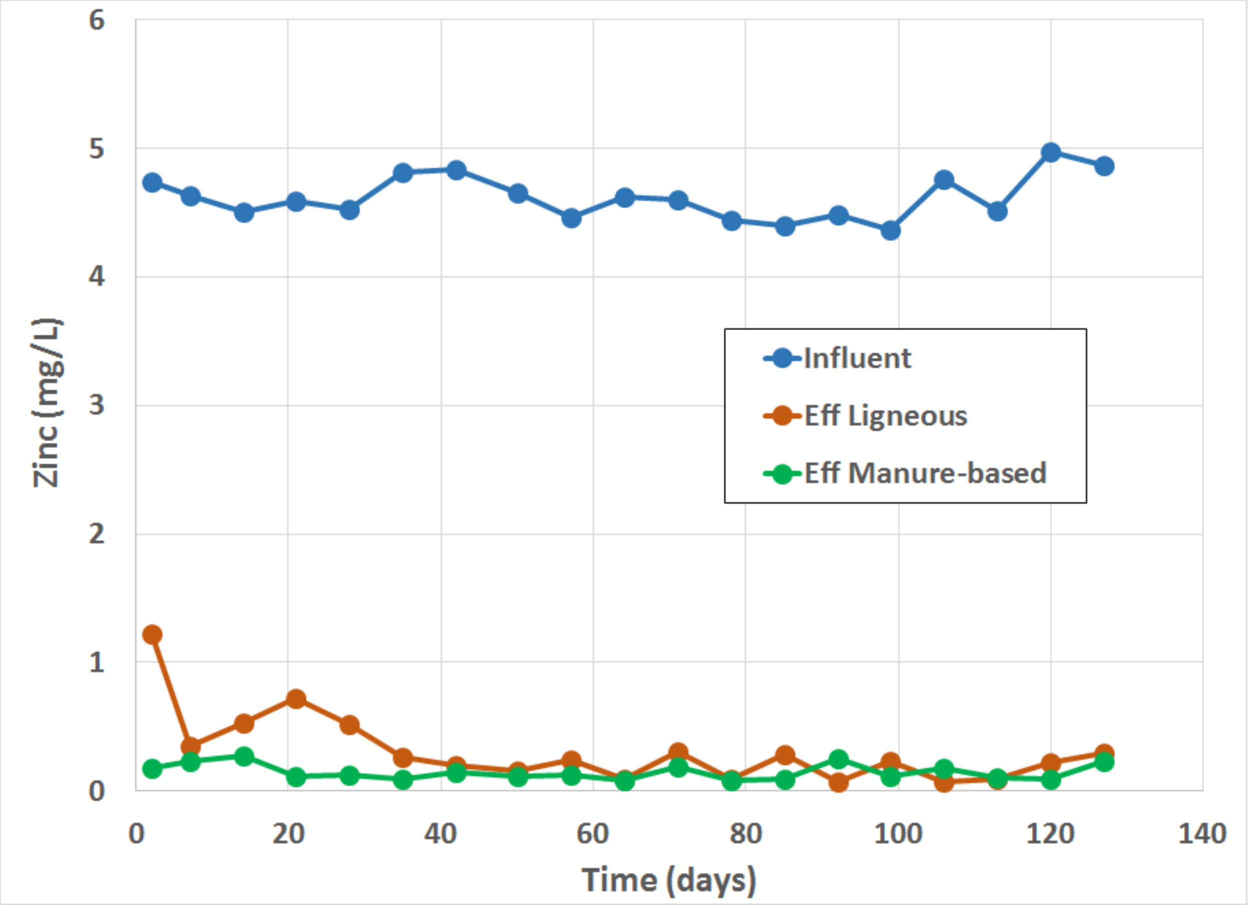


Figure 3.11 Weekly Zinc Measurement

The Haystack Creek Mine influent water had an average of 4.62 mg/L of Zinc and was consistently removed during the experimental period in both columns. The effluent average in the ligneous substrate column was higher in the initial 6 weeks, but was at the same levels as the manure-based substrate column after that. The average Zn effluent concentrations in the last 8 weeks of operations were 0.17 mg/L for the ligneous substrate column and 0.14 mg/L for the manure-based substrate column. Overall, the ligneous substrate column had a 93.2% Zn removal rate while the manure-based substrate column had a 96.8% Zn removal rate.

3.1.7 Elemental Composition of the Solid Residues

The solid substrates of the Haystack experimental columns were dried and acid digested to determine their elemental composition (Table 3.1). The elements with higher content were Ca, S, Mg, Al, Mn, Si, and Zn. The high amounts of Zn found in the columns, along with the S content, confirm that one of the main metal removal mechanisms was precipitation as sulfides, with ZnS as the potential precipitate.

Table 3.1 Elemental composition of the experimental columns solid residues.

Element	Units	Haystack Ligneous Top	Haystack Ligneous Middle	Haystack Ligneous Bottom	Haystack Manure-based Top	Haystack Manure-based Middle	Haystack Manure-based Bottom
Al	mg/kg	1450	3320	1830	2750	4460	6290
As	mg/kg	1.52	1.74	1.55	<0.716	6.18	5.26
Ba	mg/kg	20.1	8.99	5.69	61.3	87.9	31.8
Ca	mg/kg	113000	28200	1900	33800	60200	19600
Cd	mg/kg	0.56	18.1	51.5	<0.0535	0.413	39.6
Co	mg/kg	1.82	35.5	10.2	2.93	3.83	36
Cr	mg/kg	4.61	<3.81	4.61	<3.81	14.7	13.2
Cu	mg/kg	24.8	25.9	113	34.5	50.7	82.3
Fe	mg/kg	1760	1330	1420	9580	6500	5720
K	mg/kg	418	316	285	599	985	911
Mg	mg/kg	1760	849	498	1980	2860	2360
Mn	mg/kg	270	119	44.5	236	370	339
Na	mg/kg	76.5	73	65.6	55.3	95.6	87.5
Ni	mg/kg	2.2	25.6	5.2	4.33	5.26	27.3
P	mg/kg	754	697	603	851	1450	1180
Pb	mg/kg	<22.8	<22.8	140	<22.8	29.8	112
S	mg/kg	2730	4470	2830	5440	3950	4980
Se	mg/kg	1.53	<1.55	1.7	<1.55	2.56	<1.55
Si	mg/kg	183	352	268	135	193	202
Sr	mg/kg	35.9	16.8	6.98	25.3	43	21.8
V	mg/kg	3.89	3.33	<2.64	<2.64	9.85	8.77
Zn	mg/kg	346	4180	2380	101	164	4410

4. Conclusions

- Both substrates were successful in Zn removal, and all other metals in the water, except Mn. Mn is known as the most recalcitrant metal. Mn does not usually precipitate as sulfide, as Zn and Cd do.
- The ligneous substrate was slightly better in Cu removal and also in providing higher porosity with time.
- The addition of limestone was effective in providing enough alkalinity to give the SRB a suitable environment for 18 weeks at an influent flowrate of 2 liters/week (0.4 g/L of treated water). But the sulfate breakthrough point was reached in the 15th week and very probably, a few weeks after that, Zn breakthrough would have followed.
- SRB treatment in the field with the Haystack Creek mine could be successful in Zn removal with the limestone and tested substrates. The substrate load needs to be adjusted depending on the operational period and other field parameters.