

EVALUATION OF METAL REMOVAL AND TOXICITY REDUCTION IN A LOW SULFATE MINE DRAINAGE BY CONSTRUCTED WETLANDS¹

Garry H. Farmer, David M. Updegraff, James M. Lazorchak, and Edward R. Bates²

Abstract: A pilot-scale demonstration using two constructed wetlands cells is being conducted to evaluate the potential removal of metal contamination, primarily zinc, from mine drainage. The drainage from the Burleigh Tunnel, Silver Plume, CO, contains low levels of sulfate (350-450 mg/L) that may limit the production of hydrogen sulfide by sulfate-reducing bacteria; thus, limiting metal removal by the system. Total metals, anions, and field parameters in the mine drainage and the constructed wetlands effluents were routinely analyzed over 10 months. In addition, the wetlands compost was analyzed for metals sulfate-reducing bacteria and acid volatile sulfides. Zinc removal in the upflow wetlands was in excess of 99 percent (average influent zinc concentration of 56.4 mg/L) during most of the 10-month period. Further, sulfate-reducing bacteria in the wetlands substrate compost ranged from 10^6 to 10^8 colony forming units per gram of compost. Finally, 48-hour toxicity testing with fish (fathead minnows) and invertebrates (*Ceriodaphnia dubia*) found that 100 percent of both wetlands effluents had no significant acute toxicity.

Additional Key Words: Burleigh Tunnel; Constructed Wetlands; Sulfate-Reducing Bacteria

Introduction

The contamination of streams, rivers, and lakes with metals originating from mining activities has become a serious environmental problem in many areas of the United States. In Colorado, the Colorado Department of Public Health and Environment estimates 1,300 miles (2,092 kilometers) of streams and rivers have been impacted by mine drainage (USGS 1994). Over the last 10 to 15 years numerous researchers have applied constructed wetlands technology to the remediation of mine drainage. Presently, constructed wetland systems (CWS) appears to be one of the few cost effective treatments available for the remediation of mine drainage. Thus, the Environmental Protection Agency is evaluating the constructed wetlands technology at the Burleigh Tunnel (Silver Plume, CO) within the Superfund Innovative Technology Evaluation (SITE) program.

In general, there are two types of constructed wetlands, free water systems (FWS) and subsurface flow systems (SFS). Free water systems are typically composed of shallow channels or ponds and remove metals by chemical oxidation followed by precipitation of the metal oxide or hydroxide. Subsurface flow systems channel the mine drainage through a porous material with a high organic content such as compost. Sulfate-reducing bacteria (SRB) within the compost produce hydrogen sulfide that reacts with the dissolved metals forming insoluble or slightly soluble metals sulfides. The metal sulfides precipitate and are filtered from the water by the compost.

The purpose of this study is to evaluate the potential of a compost subsurface flow CWS to remove metal contamination from mine drainage containing a low to moderate concentration of sulfate (350-450 mg/L). Additionally, both effluents are being tested to determine how effective the constructed wetlands remove acute toxicity.

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Experimental Procedures and Materials

Two parallel CWS treatment cells are located adjacent to the Burleigh adit between a compressor building and an old mill. Each cell covers approximately 0.05 acres and differ in flow configuration. The cell nearest the adit is an upflow system and the other cell downflow. The flow to the CWS cells is regulated by a pair of v-notch weirs, one for the influent and one for the effluent. Each cell is designed to treat 7 gallons per minute (gpm) or a total flow of 14 gpm.

Previous construction near the Burleigh adit required the upflow cell to be 5 percent smaller (by volume) than the downflow cell. The top of the downflow cell is 51.75 feet in length and 33 feet in width. The top of the upflow cell is 69 feet long and 25.5 feet in width on the ends of the cell and 25 feet in the center. The depth of compost in each cell is 4 feet. The following table compares the dimensions and capacities of the upflow and downflow cells:

	Length	Width	Depth of Compost	Estimated Total Volume of Compost
Upflow Cell	69 feet	33/25 feet	4 feet	212 cubic yards
Downflow Cell	62 feet	33 feet	4 feet	223 cubic yards

The Burleigh drainage is collected 50 feet upstream of the adit where a sandbag dike has been constructed. The dike provides additional head to drive the drainage through the treatment cells. Once collected the drainage is transferred to an adjustable 60-degree v-notched weir via an insulated 8-inch polyvinyl chloride (PVC) pipe. The influent v-notched weir controls the flow to the system influent pipeline. The demonstration scale CWS is designed to treat 14 gpm. A rectangular weir discharges the remaining drainage into an overflow pipeline to Clear Creek without treatment.

Figure 1 provides cross-section schematic of the upflow and downflow cells. The base of each cell is made up of a gravel subgrade, a 16 oz. geofabric, a sand layer, a clay liner, followed by a high density polyethylene liner. The base is separated from the influent (or effluent) piping by a GEONET. Geofabric (7 oz) separates the perforated piping from the compost. In the upflow cell the compost is held in place on the upper level with a combination of geofabric (7 oz) and GEOGRID. In addition, the effluent (or influent) piping is supported by the GEOGRID. Above the perforated piping are 4 to 6 inches of dry compost.

Distribution piping and the geonet ensure even distribution of influent into the treatment cells and prevent short circuiting through the cells. The influent will seek a path of least resistance and will fill the distribution piping first, then the geonet, then be forced through the compost. Influent and effluent distribution piping are staggered vertically as an additional precaution to avoid short circuiting.

The compost or substrate material is composed of a mixture of 95 percent processed manure (produced from cattle manure and unidentified paper products) and 5 percent hay. The compost-hay mixture was identified as the most effective media in removing zinc from the drainage during a bench-scale test conducted by Camp, Dresser, and McKee (CDM 1993).

Influent water samples were collected from the influent wier receiving the mine drainage. Constructed wetlands effluent samples were collected at the effluent water wier with 1-liter polyethylene dippers and transferred into the sample containers. Both influent and effluent samples were collected every 2 weeks. Substrate samples were also collected with 1-liter polyethylene dippers, monthly for microbial analysis and quarterly for metals analyses. The dipper was inserted into the substrate as far as possible, typically between 2 and 2.5 feet (.62 and .77 meters).

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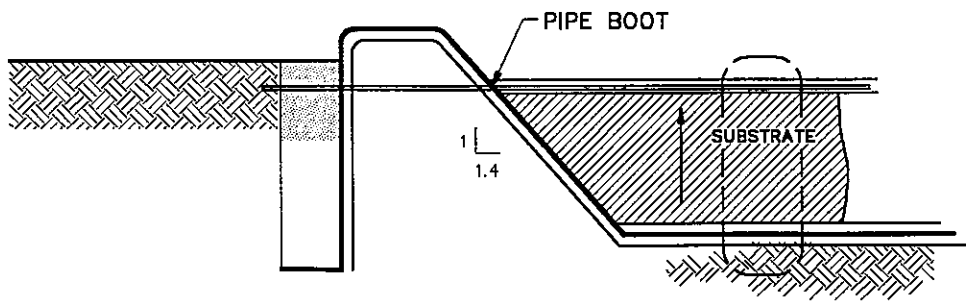
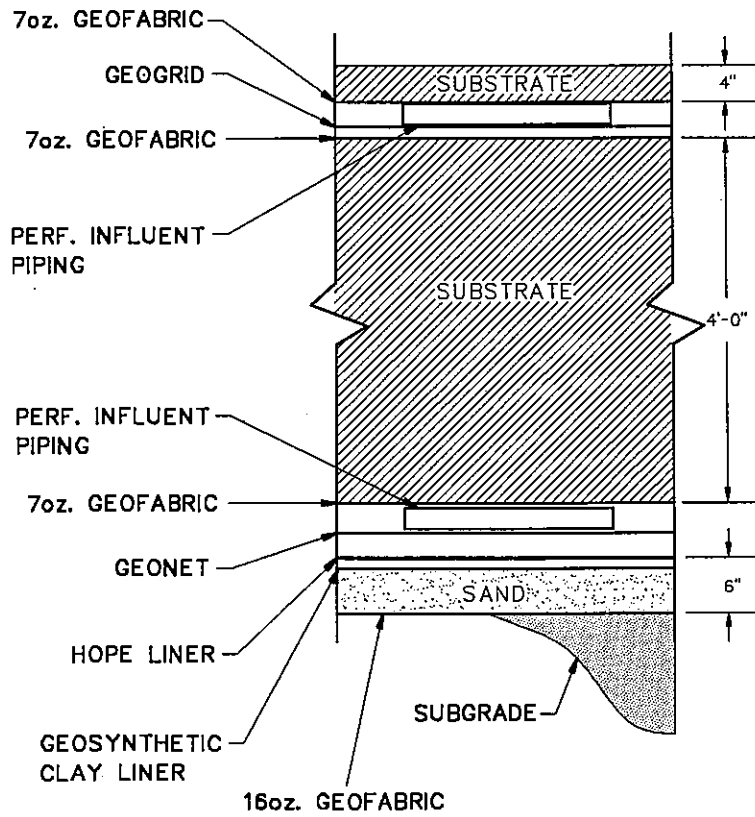


Figure 1. Schematic Cross Section of Upflow Pilot Cell

All influent and effluent water samples and substrate (metals data not presented) samples were analyzed for total metals, by inductively coupled plasma emission spectroscopy (ICP) or inductively coupled plasma mass spectrometry (ICPMS) using EPA protocols. Anion analyses were also conducted by EPA protocols using gravimetric and spectroscopic techniques for alkalinity, sulfate, nitrate, nitrite, chloride, fluoride, and sulfide (effluent only). Field measurements included the determination of pH, Eh, dissolved oxygen, and conductivity in influent and effluent water.

Table 1 provides a summary of EPA toxicity testing procedures (U.S. EPA, 1993). The test methods consist of 48-hour static-renewal acute toxicity tests using less than 24-hour-old *Ceriodaphnia dubia* and 2- to 7-day-old fathead minnow larvae (*Pimephales promelas*). Influent and effluent samples were tested using 5 dilutions and a control.

In addition, substrate samples were analyzed for sulfate-reducing bacteria by a direct counting procedure using serial dilutions of the substrate sample into 10-ml deep test tubes containing a lactate (Modified Media E, Postgate 1984) medium. Finally, enrichment cultures of sulfate-reducing bacteria were prepared from deep tube cultures by removing individual black colonies from the medium with a sterile pipette and transferring to fresh lactate medium.

TABLE 1. STANDARD OPERATING PROCEDURES FOR FATHEAD MINNOW ACUTE TOXICITY TESTS FOR SUPERFUND SAMPLES

Test Criteria	Specifications
Test Type	Static-renewal
Test Duration	48 hour
Temperature	20°C ± 1°C
Photoperiod	16 hr. light/8 hr. dark
Test Chamber Size	175 ml (plastic cups)
Test Solution Volume	150 ml
Renewal of Test - solution	Daily
Age of Test Organisms	3 to 7 days ± 24 hr. age range
Number of Organisms/per test chamber	10
Number of Replicate-Chambers/Conc.	2
Number of Organisms/Concentration	20
Feeding	Feed newly hatched brine shrimp prior to testing. Do not feed during the test.
Dilution Water	Moderately Hard Reconstituted Water
Endpoint	Mortality
Test Acceptability	≥90% survival in the controls
Endpoint	LC50

Results

Table 2 contains influent and effluent sample results from both constructed wetlands cells. The influent analyses indicate the primary metals contained in the Burleigh drainage are calcium (85-95 mg/L), magnesium (40-50 mg/L), zinc (45-65 mg/L), sodium (9.0-15 mg/L), potassium (2.9-3.5 mg/L), and manganese (2.0-2.6 mg/L). The remaining metals analyzed during the study, aluminum, arsenic, cadmium, iron, lead, nickel, and silver were detected in concentrations less than 0.5 mg/L in influent samples.

Initially, the upflow constructed wetlands effluent samples contained elevated levels of potassium (214 mg/L), sodium (33 mg/L), magnesium (73 mg/L), and calcium (88 mg/L) that decreased over the first 10 months of the study. Zinc in the upflow cell effluent ranged from 0.16 to 6.8 mg/L, manganese from 0.058 to 2.7 mg/L, and nickel from 0.0062 to 0.018.

The downflow cell effluent also contained elevated levels of potassium (43 mg/L) and sodium (19 mg/L); however, concentrations of magnesium and calcium were similar to influent levels. Zinc in the effluent of the upflow cell ranged from 0.42 to 2.4 mg/L, manganese from 0.17 to 0.73 mg/L, and nickel from 0.13 to 16.4 mg/L.

Somewhat elevated levels of chloride, sulfate, phosphorus and ammonia were also present in the effluent of each cell at startup; however, their concentrations decreased substantially after two months of operation. Initially, the concentration of sulfate in the upflow cell effluent was 357 mg/L, but rapidly dropped to a low of 278 mg/L followed by a gradual increase and stabilization between 380 to 393 mg/L. Sulfate concentrations in the downflow cell were 350 mg/L at startup but dropped to 275 mg/L and also gradually increased to a somewhat stable level between 330 to 365 mg/L. Sulfide levels in the effluents of cell ranged from 0.18 mg/L in the upflow cell to a maximum of 5.6 mg/L in the upflow cell and 7.8 mg/L in the downflow cell. The sulfide concentrations of both systems is greater in the spring and fall and at a minimum during the summer.

Tables 3 and 4 show results of toxicity testing with Ceriodaphnia dubia and Fathead minnows conducted on wetlands effluent and Burleigh drainage samples collected after seven months of operation. The results indicate that 50 percent of the Ceriodaphnia dubia are killed in a solution containing 0.31 percent of the mine drainage. A 50 percent mortality of the Fathead minnows was observed in a solution containing 0.73 percent Burleigh drainage. The survival of Ceriodaphnia dubia in 100 percent downflow effluent was 15 of 20 organisms and 16 of 20 Fathead minnows. Survival rates in 100 percent of the upflow effluent were 15 of 20 Ceriodaphnia dubia and 20 of 20 Fathead minnows. Survival in both effluents was not significantly different from the control at an alpha level of 0.5.

Results of serial dilutions of substrate samples collected monthly found sulfate-reducing bacteria at concentrations ranging from 10^4 and 10^8 colony forming units per gram of substrate (wet). Generally, counts of sulfate-reducing bacteria present in the upflow cell were 10^7 while counts in the downflow cell were 10^4 to 10^5 . In addition, sulfate-reducing bacteria counts decreased in both cells during November and December.

Discussion

Previous work with SFS wetlands and bioreactors containing sulfate reducing bacteria (Eger et al. 1993, Hammack et al. 1994, Fyson et al. 1994, and Wildeman et al. 1992) have found that arsenic, cadmium, copper, lead, iron, nickel, and zinc are removed as sulfides or coprecipitate with sulfide precipitation. The comparison of effluent to influent concentrations (Table 2) during this study indicate zinc, arsenic, cadmium, nickel, and silver were removed by the compost and hay wetlands. The removal of arsenic, cadmium, and nickel is significant because these metals are present in low concentration in the Burleigh drainage.

Table 2. Analytical Results for Influent in mg/L

Analyte	Sample Feb.	Sample March	Sample April	Sample May	Sample June ¹	Sample July	Sample August	Sample Sept.	Sample October	Sample Nov.	Sample Dec.
Metals:											
Aluminum	ND	ND	ND	0.045	0.068	ND	ND	ND	ND	ND	0.04
Arsenic	ND	0.004	0.013	0.056	ND	ND	ND	ND	ND	ND	ND
Cadmium	0.12	0.10	0.10	0.090	0.088	0.092	0.098	0.092	0.11	0.10	0.089
Calcium	91	88.2	94.3	86.6	87.6	92.8	93.6	90.0	92.5	89.2	96.8
Iron	0.3	0.22	0.34	0.26	0.33	0.26	0.24	0.29	0.28	0.28	0.36
Lead	0.01	0.014	0.015	0.015	0.018	0.15	0.015	0.016	0.014	0.014	0.016
Magnesium	45	43.2	45.4	48.1	48.1	47.4	47.9	46.6	47.0	46.2	48.3
Manganese	2.4	2.4	2.6	2.0	2.0	2.2	2.4	2.3	2.4	2.2	2.5
Nickel	0.06	0.040	0.040	0.039	0.039	0.044	0.044	0.044	0.049	0.051	0.047
Potassium	3	2.9	3.0	3.4	3.3	3.0	3.0	3.5	3.0	2.9	3.0
Silver	ND	ND	0.0001	0.00011	0.00021	0.00011	0.00011	0.00041	ND	ND	.0004
Sodium	14	9.0	10.0	2.2	2.9	2.5	4.8	2.3	12.3	14.8	16.8
Zinc	59	56.7	62.0	50.4	49.6	58.0	56.1	57.0	58.6	56.5	62.9
Anions:											
Sulfate	390	380	386	316	368	387	388	410	404	410	412
Sulfide, Total	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Fluoride	1.1	1.1	1.1	1.0	0.94	1.0	1.1	1.0	1.0	1.0	11.0
Chloride	21	20.8	22.1	17.0	17.6	18.1	19.1	19.9	19.6	20.1	21.3
Phosphorus, Total	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Orthophosphate	ND	0.3	ND	0.40	0.25	0.077	ND	ND	ND	0.13	0.24
Nitrite as N	NA	ND	0.08	ND	ND	ND	ND	ND	ND	ND	ND
Nitrate Plus Nitrite	ND	ND	0.08	ND	ND	2.0	1.8	ND	ND	ND	ND
Nitrate as N	NA	ND	ND	ND	ND	2.0	1.8	ND	ND	ND	ND
Ammonia	0.12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total Solids:											
TSS	ND	12.8	17.8	7.9	10.5	9.4	10.4	13.0	16.4	8.0	13.4
TDS	680	694	652	632	679	696	731	717	695	709	699
TOC	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Alkalinity:											
As CaCO ₃	120	104	106	106	107	106	104	102	107	82.4	101.3

ND Not detected
¹ average of 3 rounds

Table 2 (Continued). Analytical Results for Downflow Effluent

Analyte	Sample Feb	Sample March	Sample April	Sample May	Sample June	Sample July	Sample August	Sample Sept.	Sample October	Sample Nov.	Sample Dec.
Metals:											
Aluminum	ND	0.021	0.028	0.028	0.023	0.014	0.016	0.038	0.022	0.023	0.019
Arsenic	ND	0.00056	0.022	0.071	0.0012	0.0011	0.0011	0.0011	0.0011	ND	ND
Cadmium	ND	0.00030	0.00041	0.00091	0.00073	ND	0.00032	ND	0.00048	0.00041	0.00059
Calcium	100	106	112	110	109	117	116	118	112	112	119
Iron	ND	0.92	1.0	1.0	0.98	1.1	1.5	1.9	1.7	1.8	2.2
Lead	0.007	0.0014	0.0011	0.0015	0.0012	ND	ND	0.0020	ND	ND	0.0064
Magnesium	56	55.8	58.4	58.0	58.9	56.9	57.0	59.8	57.2	58.0	59.3
Manganese	1.6	1.4	1.4	1.4	1.5	1.8	2.2	2.1	1.8	1.6	1.6
Nickel	ND	0.0077	0.0093	0.0089	0.014	0.011	0.014	0.016	0.022	0.020	0.018
Potassium	43	55.8	52.3	43.9	25.4	19.3	22.4	25.0	21.0	16.8	12.3
Silver	ND	0.00081	0.00008	0.0026	0.00010	0.00025	0.00014	0.00033	0.00064	ND	0.00022
Sodium	19	17.6	16.7	17.0	14.4	14.6	15.6	15.4	14.8	15.5	14.8
Zinc	1.3	9.7	15.4	11.5	10.2	15.9	14.9	16.4	14.8	12.1	10.2
Anions:											
Sulfate	350	354	338	275	330	346	328	349	342	365	391
Sulfide, Total	NA	4.6	3.9	2.0	1.7	4.1	3.0	2.7	7.8	7.4	4.4
Fluoride	0.9	0.88	0.89	0.89	0.84	1.0	1.0	.095	0.88	0.87	1.0
Chloride	23	22.0	27.6	19.7	19.1	18.8	20.2	21.6	20.8	20.8	21.6
Phosphorus, Total	0.97	10.2	10.9	10.9	9.6	8.6	9.6	9.1	9.0	8.2	7.2
Orthophosphate	0.65	11.5	10.9	10.8	9.2	8.0	7.3	11.2	8.5	8.8	5.6
Nitrite Plus Nitrite as N	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Nitrite as N	ND	0.24	ND	ND	ND	2.3	1.8	ND	ND	ND	ND
Nitrate as N	NA	0.24	ND	ND	ND	2.3	1.8	ND	ND	ND	ND
Ammonia	5.8	5.8	5.8	4.5	3.0	3.0	3.2	2.6	2.6	1.5	1.2
Total Solids:											
TSS	18	39	43.1	3.8	24.6	44.3	46.0	48.4	46.0	40.0	34.5
TDS	730	822	774	746	746	734	736	740	706	734	756
TOC	ND	40.5	28.6	23.6	24.0	15.6	14.7	11.3	9.0	5.0	13.0
Alkalinity:											
As CaCO ₃	190	201	206	194	197	189	192	189	187	152	148

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ND Not detected
¹ average of 3 rounds

Table 2 (Continued). Analytical Results for Upflow Effluent Samples

Sample	Sample Feb.	Sample March	Sample April	Sample May	Sample June	Sample July	Sample August	Sample Sept.	Sample October	Sample Nov.	Sample Dec.
Metals:											
Aluminum	NA	0.14	0.23	0.045	0.034	0.038	0.019	0.038	0.016	0.025	0.014
Arsenic	NA	0.0066	0.032	0.076	ND	ND	ND	ND	0.0011	0.0011	0.0012
Cadmium	NA	0.00046	0.00034	0.00022	ND	ND	ND	ND	ND	ND	ND
Calcium	NA	88.5	114	119	119	132	133	129	128	122	125
Iron	NA	0.50	0.74	0.26	0.34	1.10	3.0	4.7	5.6	7.0	7.2
Lead	NA	0.0036	0.012	0.0018	0.0020	ND	ND	0.0013	ND	ND	ND
Magnesium	NA	73.0	66.2	63.0	62.6	60.0	57.7	55.0	54.3	52.5	51.9
Manganese	NA	0.058	0.112	0.21	0.56	1.5	2.2	2.5	2.6	2.7	2.9
Nickel	NA	0.0062	0.0090	0.0086	0.012	0.0064	0.0083	0.0116	0.017	0.018	0.017
Potassium	NA	214	129	70.3	30.1	15.5	12.0	9.6	9.8	11.6	7.6
Silver	NA	0.0008	0.00028	0.00007	0.00014	0.00015	0.00021	0.00029	0.00052	ND	0.0003
Sodium	NA	33.4	24.6	2	15.3	14.6	14.4	13.9	14.2	14.2	15.0
Zinc	NA	0.16	0.28	19.4	0.24	0.24	0.48	1.11	2.8	6.8	8.4
				0.23							
Anions:											
Sulfate	NA	357	354	278	344	364	380	393	380	371	377
Sulfide, Total	NA	4.1	5.6	1.4	2.9	1.0	0.84	0.18	3.2	3.8	3.9
Fluoride	NA	0.44	0.67	0.80	0.79	0.90	1.0	1.0	0.97	1.1	1.2
Chloride	NA	78.6	54.8	28.6	21.6	19.6	20.2	20.8	20.8	23.2	22.3
Phosphorus, Total	NA	23.8	20.6	18.0	18.3	12.0	10.2	4.8	7.4	6.2	6.1
Orthophosphate	NA	26.8	20.8	17.2	16.8	15.0	8.5	9.3	7.0	5.9	3.7
Nitrite Plus Nitrite as N	NA	ND	ND	ND	ND	ND	0.077	ND	0.018	0.16	ND
Nitrite as N	NA	ND	0.060	ND	ND	1.9	1.80	ND	ND	ND	ND
Nitrate as N	NA	ND	0.060	ND	ND	1.9	1.80	ND	ND	ND	ND
Ammonia	NA	21.7	14.0	8.6	4.4	2.8	1.40	0.94	0.76	0.38	1.0
Total Solids:											
TSS	NA	9.0	15.6	ND	3.2	10.4	17.4	28.2	39.2	52.0	46.0
TDS	NA	1,295	1,060	869	802	791	784	288	742	727	729
TOC	NA	158	54.6	29.7	17.8	9.3	7.4	6.1	6.4	9.4	12.0
Alkalinity:											
As CaCO ₃	NA	357	309	248	234	208	188	170	166	141	150

ND = Not detected
¹ average of 3 rounds

Table 3. Toxicity Results from Ceriodaphnia Dubia

Sample	Concentration	Survival	LC50	Limits
Upflow Cell Effluent	Control	20/20	NA	
	50%	20/20		
	75%	20/20		
	100%	15/20		
Downflow Cell Effluent	Control	20/20	NA	
	6.25%	19/20		
	12.5%	20/20		
	25%	19/20		
	50%	16/20		
	100%	15/20		
Burleigh Drainage	Control	20/20	0.31%	(0.26-0.36)
	0.094%	19/20		
	0.19%	19/20		
	0.375%	5/20		
	0.75%	0/20		
	1.5%	0/20		

Table 4. Toxicity Results of Fathead Minnows (Pimephales Promelas)

Sample	Concentration	Survival	LC50	Limits
Upflow Cell Effluent	Control	20/20	NA	
	50%	20/20		
	75%	20/20		
	100%	20/20		
Downflow Cell Effluent	Control	20/20	NA	
	6.25%	20/20		
	12.5%	20/20		
	25%	19/20		
	50%	19/20		
	100%	16/20		
Burleigh Drainage	Control	20/20	0.73%	(0.60-0.88) ¹
	0.19%	20/20		
	0.375%	15/20		
	0.75%	14/20		
	1.5%	0/20		
	3.0%	0/20		

Figure 2 shows the percent removal of zinc in both cells over the first 10 months of the study. The pattern observed for the downflow cell, a high initial removal followed by a steep drop in removal efficiency followed by a gradual increase of zinc removal was observed in constructed wetlands studies reported by Machamer and Wildeman (1992). They suggest the initial high removal phase results from sorption of the metals to the compost, and once sorption sites are filled, the removal efficiency drops. Gradually, sulfate-reducing bacteria become established and metal removal reflects the growing population of sulfate-reducing bacteria.

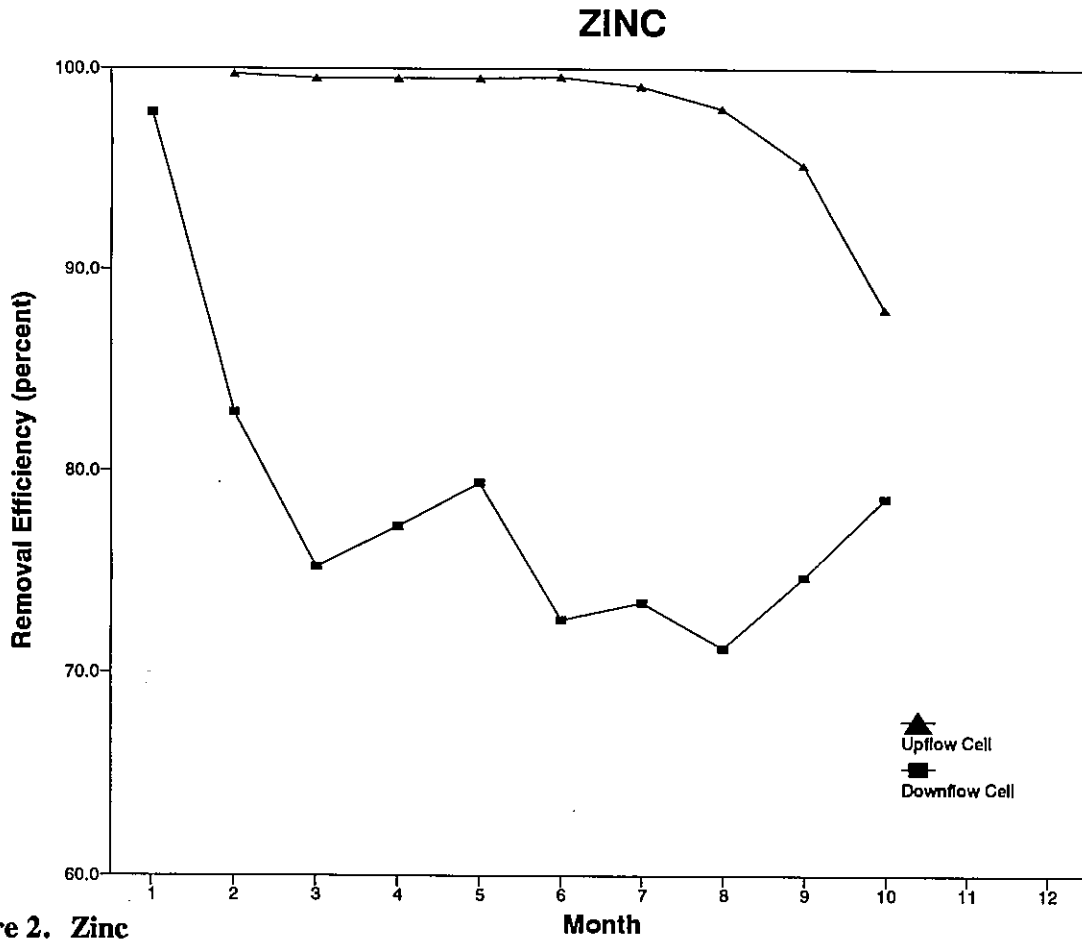


Figure 2. Zinc

Zinc removal in the downflow cell was consistently between 70 and 80 percent during the summer and fall. The level of zinc removal is lower than observed by Machamer and Wildeman (1992) during similar studies conducted at the Big 5 (Idaho Springs, Colorado). In addition, substrate samples collected from the downflow cell contained lower numbers of sulfate-reducing bacteria compared to the upflow cell. Further, the downflow cell substrate did not appear to accumulate significant amounts of the fine black particles, assumed to be metal sulfates, as the upflow cell substrate. Thus, another process, such as zinc carbonate precipitation, may contribute to the removal of zinc in the downflow cell. Zinc removal in the upflow cell was consistently greater than 90 percent over the first 8 months. However, a gradual decline in zinc removal, to a low of 88 percent, was observed during November. The decline in zinc removal efficiency paralleled a drop in sulfate-reducing bacteria counts observed in substrate samples collected from the upflow cell. The lower counts of sulfate-reducing bacteria observed in early winter may be related to lower levels of lactate present in the substrate. Lower lactate levels could result from decreased rates of substrate utilization and metabolism by single and multicellular microorganisms or bacteria.

Manganese was not consistently removed from the mine drainage by either cell. Figure 3 shows the percent removal of manganese in both wetlands over the first 10 months of the study. Removal of manganese by the upflow cell was initially 98 percent; however, removal decreased to 8 percent after 7 months of operation. The downflow cell showed a gradual decrease in performance removing an average of 32 percent of the manganese between months 1 and 6 followed by a slight increase. Manganese is not expected to form a sulfide at the pH or Eh conditions in the cells; however, there is sufficient carbonate in these systems that $MnCO_3$ (rhodochrosite) may precipitate.

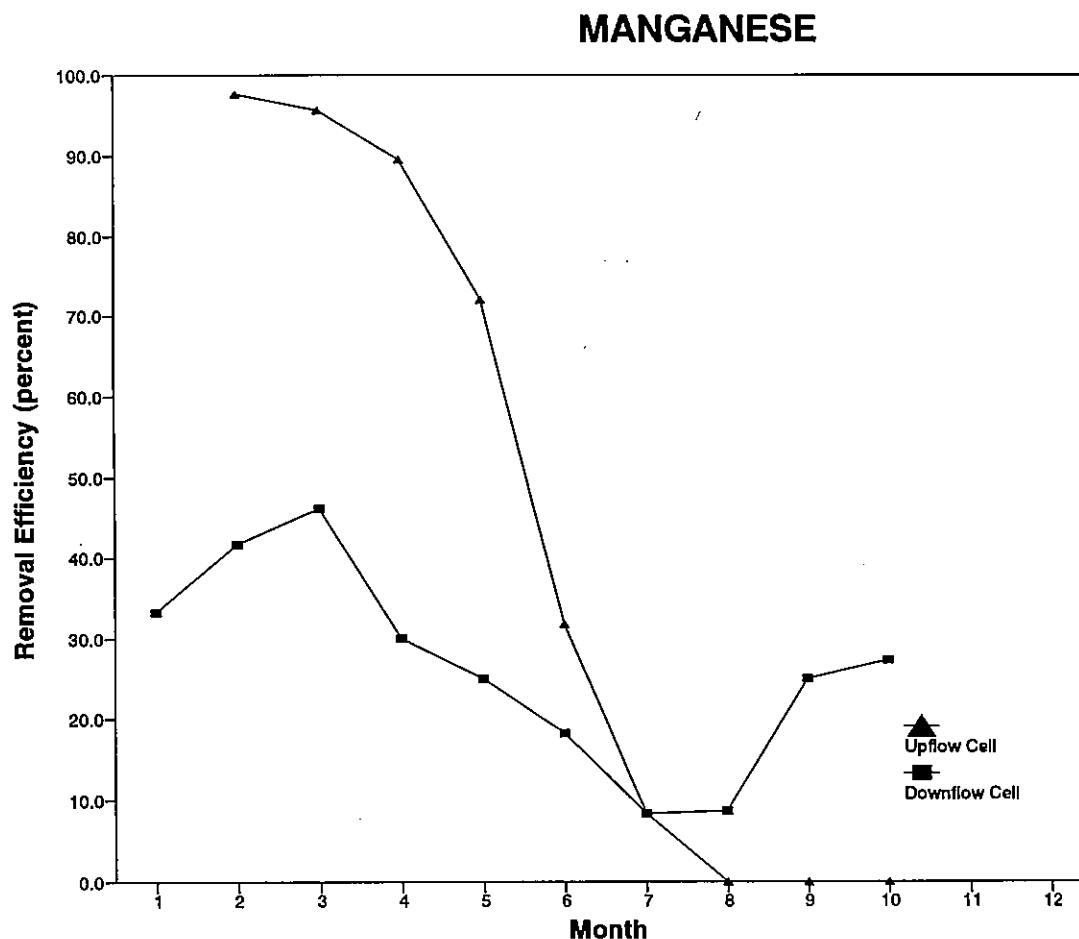


Figure 3. Manganese

Toxicity testing results of the downflow and upflow effluent indicate no significant acute toxicity was present. The zinc concentration was reduced to 16.4 mg/L in the downflow cell, a 71% reduction from the influent concentration and to 1.1 mg/L in the upflow cell, a 98% reduction. The influent LC50 for *Ceriodaphnia* was 0.31% which would indicate that 0.180 mg/L of zinc was toxic in the effluent. The higher zinc levels of 16.4 mg/L and 1.1 mg/L in the effluents would indicate that most of the zinc is bound up with organic and/or inorganic material rendering it not biologically available to the test organisms. Additionally, toxicity testing of the wetlands effluents will be performed in the winter, spring and summer of 1995 to evaluate if changes in toxicity level and/or...

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