Iron removal in a simulated wetland for acid mine drainage treatment.

Lois E. McHerron, S. Edward Stevens, Jr., Harold J. Webster, Lloyd R. Stark, and Kim Dionis

The Pennsylvania State University, University Park, PA

ABSTRACT

Three moss species and one alga were tested for their effectiveness in removing iron in a simulated wetland. Two experiments were conducted with low and high initial iron concentrations. Water was collected at the outlet of each lane for metal analysis. in the second experiment (high iron), water samples were also taken from four locations within each lane. After three months of exposure to iron, plant material was collected for determination of iron content in the tissues and total bacterial counts. In the first experiment (low iron), Oedogonium. sp., Drepanocladus fluitans, and Sphagnum recurvum, reduced the iron concentration to zero within 2 h. Pohlia nutans reduced the iron concentration by 50%. In the second experiment, Sphagnum was the most effective followed by Drepanocladus and Pohlia, with all species reducing the iron concentration by at least 70%. most iron removal occurred in the upper half of the lane. Only Sphagnum did not show signs of saturating. The plant tissues contained significant amounts of iron indicating that they absorbed much of the iron that was removed. In Sphagnum and Pohlia, the brown portion contained most of the iron. The iron content of Sphagnum. decreased dramatically along the length of the lane. High numbers of heterotrophic and iron-oxidizing bacteria were found in all lanes, but their numbers did not appear to be associated with effectiveness of iron removal. The bacterial species diversity was similar to that found in AMD wetlands. This study indicates that mosses can remove significant amounts of Fe from water before saturating. Microbes which can also remove iron are associated with the plants.

INTRODUCTION

A number of volunteer and man-made wetlands in Pennsylvania and Ohio are currently being used to treat acidic mine drainage (AMD). Many of the man-made wetlands have been constructed based on limited knowledge on how the wetlands function. Several theories have been proposed to explain how metal removal and changes in pH occur. These include sulfate reduction (Wieder and Lang 1982), cation exchange (Burris et al. 1984), and microbial oxidation (Stone 1984; Gerber et al. 1985). As the use of wetlands as a treatment system increases, the importance of knowing which mechanisms are most effective in removing metals becomes more critical.

At Penn State University, we have begun a screening program to test the effectiveness of various plant species (mostly bryophytes and algae to date) in removing iron (Fe) from water. Using the greenhouse simulated wetland developed by Burris et al. (1984), the plants may be grown hydroponically and exposed to varying metal concentrations and pH, thereby modeling a field wetland treatment system.

The objectives of this study are: 1) to evaluate the capacity of bryophytes and algae to remove Fe and raise the pH, 2) to determine the amount of Fe taken up by the plant during exposure to Fe, and 3) to identify the microbial populations associated with metal removal. This paper reports our findings from the first set of species tested in the simulated wetland.

METHODS

Simulated Wetland Experiments

Experiments were conducted on the simulated wetland in the Buckhout greenhouse at The Pennsylvania State University (Burris et al. 1984). The four lanes of the wetland were planted with three species of mosses and one alga in early Sept 1986. Lane 1 contained Oedogonium sp. (a filamentous green alga) and was maintained at a pH of 6.0. Oedogonium was collected from an abandoned strip mine in Clarion Co., PA. Lane 2 contained Drepanocladus fluitans and was maintained at pH 5.2. This species is an aquatic moss known to absorb metal ions; plants were collected from McKean Co., PA. Lane 3 contained Sphagnum. recurvum collected from McKean Co., PA and was maintained at pH 5.2. This species of peat moss has been shown to remove Fe from mine water (Burris et al. 1984). Lane 4 contained Pohlia nutans at pH 2.8. Pohlia is an upright, turf-forming subaquatic moss that can tolerate low pH and water high in Fe and Mn (Webster 1985). Plants were collected from a coal seam roadcut along Interstate 80, Clearfield Co., PA. The maintenance pHs were selected based on the pH at which the organisms were collected in the field.

The pH of the reservoir buckets were maintained daily by addition of H_2SO_4 or NaOH. Water recirculated through the lanes at a flow rate of 60 ml/min. After a period of acclimation for the plants (1 wk), $FeSO_4$ and water, as necessary, was added every three days to each reservoir to give a concentration of approximately 10 mg/l. Three days was the length of time required for the concentration in the reservoir to be reduced to zero.

At the beginning of an experiment the simulated wetland was drained, the reservoir buckets filled with tap water, and the pH of each reservoir adjusted. A given amount of $FeSO_4$ was dissolved in each reservoir. The water was then pumped through each lane and collected at the outlet where water samples were collected hourly. Flow rate and pH for each lane were also monitored hourly. Water samples were periodically taken from each reservoir to detect changes in inlet concentrations during the course of the experiment. All water samples were immediately filtered through 0.45 mm filters and acidified to a pH of less than 2.0 with 50% HNO_3.. They were stored at 2^0C until Fe concentrations could be determined using a Buck Scientific 200 Atomic Absorption Spectrophotometer.

Experiment No. 1 was conducted for 18.5 h on 30 Oct 1986 with an initial Fe concentration of 11-14 mg/l. Experiment No. 2 was conducted on 4 Dec 1986 with an initial Fe concentration of 39-43 mg/l. During this experiment additional water samples were collected with a pipette from points 2 (A), 4 (B), 8 (C), and 12 (D) ft down the length of each lane to determine the gradient of Fe removal within each lane. Between experiments the simulated wetland was maintained as described above. As a result, plants were exposed to dissolved Fe between experiments. Since it was difficult to keep the Fe in solution at the pH of Lane 1 (6.0), the pH of this lane's reservoir was gradually lowered to 5.5 following the first experiment. In Experiment No. 2, therefore, Lane 1 was run at this lower pH.

Plant Tissue Analyses

On 8 Dec 1986 samples of vegetation were collected from locations A, C, and the end of each lane for the purposes of determining the concentrations of Fe in the plant tissues. Four-inch wide sections of plant material across the entire width of each lane were obtained. These plant samples were stored in plastic bags at 20C until they could be processed.

Plant material was washed in running tap water until the water ran clear. The Sphagnum and Pohlia were separated into brown and green portions. Plant samples were dried in paper bags at 75-80^{0}C for 48 h, then ground in a Wiley Mill with a 20 mesh screen. Approximately 500 mg of ground plant material was acid digested with 5 ml distilled water, 10 ml concentrated HNO_3, and 5 ml concentrated H_2SO_4 at 160^{0}C for 30 min. Samples were cooled before filtering through Whatman GFC filter paper. Filtered samples were then diluted to a volume of 100 ml with distilled water and stored at 2^0C. Fe analyses were done on the atomic absorption spectrophotometer.

Bacterial Cultures

On 8 Dec 1986, 3 cm diameter cores were taken from locations A, C, and the end of each lane for total bacterial counts and bacterial species diversity. Cores were returned to the lab and separated into green and brown portions for the Sphagnum. and Pohlia. Composite samples of the Oedogonium and Drepanocladus were used. Subsamples (approx. 2.5 g) were blended in a Waring blender with 1% sodium pyrophosphate to release the microorganisms. Suspensions were immediately diluted to a series of 1:100, 1:1000, 1:10000, and 1:100,000 in sterile distilled water. Each dilution was plated in triplicate on six different types of agar media. An Fe-supplemented inorganic media was used to encourage growth of Fe-oxidizing microbes. Three media with low, medium, and high concentrations of organics were used to grow heterotrophic bacteria. Finally, two levels (medium and high) of a protein-based medium were used for growing heterotrophs which prefer protein for their nutrition. All plates were surface inoculated and incubated at 27^0C.

Colonies were counted after 10 days on plates on which 30-300 colonies had developed. The counts included fungi, actinomycetes, and bacteria. The presence of Fe oxidizers was determined visually by the formation of a deep blue color after flooding the plate with 0.2% tetramethyl benzidine in 2M acetic acid. Colony types were distinguished on the basis of relative size, color, conformation, border, and elevation.

RESULTS AND DISCUSSION

Experiments

In Experiment No. 1, the inlet water remained at approximately 14 mg Fe/l for the duration of the experiment except for Lane 1. After 11 h the Fe concentration in the reservoir of Lane 1 had dropped to 4.7 mg/l. This drop was attributed to precipitation of the Fe at the high pH (6.0). Within the first 1.5 h, the Fe concentration at the outlets of Lanes 1, 2, and 3 decreased to 0 mg/l and remained at that level for the duration of the experiment (Table 1). In Lane 4, the Fe concentration gradually decreased to 6.0 mg/l over the first 7.5 h, then gradually increased to 8.1 mg/l by 18.5 h. These results indicated that Drepanocladus and Sphagnum. were very effective in removing Fe at low concentrations. It is unclear from the data whether Oedogonium was also effective in removing Fe or whether the decrease in Fe concentration in the outlet was due to precipitation. Pohlia plants reduced the Fe concentration by 50%.

Cation exchange is believed to be a primary mechanism for metal removal by plants. Since the Fe concentration in Lane 4 began to increase after an initial decrease, the cation-exchange sites on the Pohlia may have become saturated. If the experiment were continued, the Fe concentration at the outlet would be expected to approach the concentration in the inlet. In Lanes 2 and 3 the pH dropped during the experiment. pH decreased during the first 4.5 h and then increased in Lanes 1 and 4. The reduction in pH is consistent with the cation-exchange theory of Fe removal in which Fe cations are exchanged with hydrogen ions. The release of hydrogen ions into the water would lower the pH.

To better assess the saturation limits of each plant species tested, a second experiment was designed with a higher initial Fe concentration (approx. 40 mg/1). Additional sampling points within each lane were included to determine if a gradient of Fe removal was present. Between experiments the *pH* of Lane I was gradually decreased to 5.5 to reduce the problem of Fe precipitation. Unfortunately the Oedogonium. did not tolerate the lower pH, and the population declined significantly before the last experiment. Lane 1 was, therefore, not included in the analysis of Experiment No. 2.

Initial Fe concentrations in the reservoirs ranged from 39.0 mg/1 to 41.5 mg/l and did not decrease significantly during the course of the second experiment. The outlet *pH* of Lanes 2 and 3 dropped dramatically in the first hour, then decreased slightly until reaching a plateau in the last 3 h (Table 2). The outlet pH of Lane 4 did not change significantly from the inlet (2.8) during the experiment.

The concentration of Fe in the outlet of Lane 2 (Drepanocladus) was initially low, but gradually increased after 6 h until plateauing at 6 mg/l after 11 h (Figure 1). A similar pattern was observed at the other

sampling points within the lane. The Drepanocladus showed reduced effectiveness in removing the Fe after 7 h in the upper 4 ft (B) of the lane. Over the next 2 h, the decline in removal occurred first at C then at D and the outlet, showing a downstream progression in saturation. The final plateau in both pH and Fe concentration indicates that cation exchange may have reached an equilibrium. After 13 h, 86% of the Fe had been removed from the water compared to an initial reduction of 94%. Nearly all of the Fe removal by the Drepanocladus occurred in the upper half of the lane (79% after 13 h). After 8 h, there was no further reduction in Fe between the middle of the lane (C) and the outlet.

The outlet Fe concentration for Sphagnum (Lane 3) gradually increased during the experiment until leveling off after 11 h (Figure 2). The pH also stabilized at this time (Table 2). Hourly changes in Fe concentrations at the other **sampling points within** the lane paralleled those at the outlet.

Table 1. Fe concentrations and pH in the outlets of each lane during the first experiment.

	Oedogonium		Drepanocladus		Sphagnum		Pohlia	
Hour	pH	mgFe/l	pH	mgFe/l	pH	mgFe/l	pH	mgFe/l
avg. influent	6.00	9.77	5.20	14.21	5.20	14.12	2.80	14.60
0	5.55	0	3.88	0	3.83	0	--	-
0.5	5.58	0.51	4.07	0	3.91	0	2.70	7.03
1	5.75	0.13	4.02	0	4.05	0	2.71	6.93
1.5	5.54	0	4.01	0	3.96	0	2.72	7.10
2.5	5.32	0	3.98	0	3.92	0	2.68	6.44
3.5	5.34	0	3.96	0	3.94	0	2.68	6.30
4.5	5.21	0	3.91	0	3.81	0	2.60	6.14
5.5	5.43	0	3.85	0	3.85	0	2.67	6.74
6.5	5.88	0	3.91	0	3.90	0	2.77	6.04
7.5	6.32	0	3.85	0	3.84	0	2.75	6.01
8.5	6.06	0	3.88	0	3.90	0	2.81	6.34
10.5	6.22	0	3.82	0	3.90	0	2.84	6.77
12.5	6.14	0	3.67	0	3.82	0	2.82	6.80
14.5	6.76	0	3.54	0	3.76	0	2.80	7.30
16.5	6.50	0	3.50	0	3.65	0	2.80	7.83
18.5	6.12	0	3.49	0	--	--	2.78	8.06

Table 1. Fe concentrations and pH in the outlets of each lane during the first experiment.

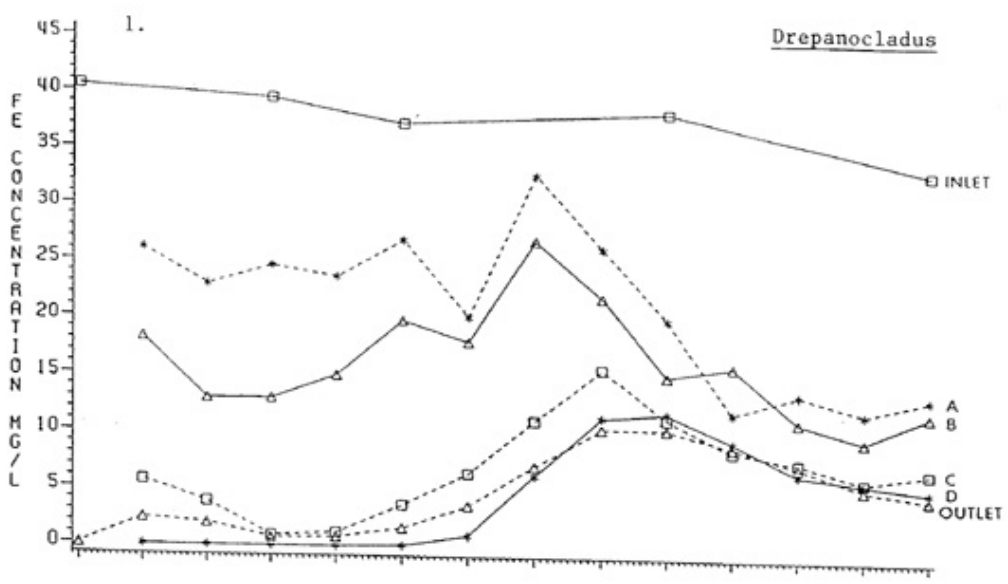
	Oedogonium		Drepanocladus		Sphagnum		Pohlia	
Hour	pH	mgFe/l	pH	mgFe/l	pH	mgFe/l	pH	mgFe/l
avg. influent	6.00	9.77	5.20	14.21	5.20	14.12	2.80	14.60
0	5.55	0	3.88	0	3.83	0	--	--
0.5	5.58	0.51	4.07	0	3.91	0	2.70	7.03
1	5.75	0.13	4.02	0	4.05	0	2.71	6.93
1.5	5.54	0	4.01	0	3.96	0	2.72	7.10
2.5	5.32	0	3.98	0	3.92	0	2.68	6.44
3.5	5.34	0	3.96	0	3.94	0	2.68	6.30
4.5	5.21	0	3.91	0	3.81	0	2.60	6.14
5.5	5.43	0	3.85	0	3.85	0	2.67	6.74
6.5	5.88	0	3.91	0	3.90	0	2.77	6.04
7.5	6.32	0	3.85	0	3.84	0	2.75	6.01
8.5	6.06	0	3.88	0	3.90	0	2.81	6.34
10.5	6.22	0	3.82	0	3.90	0	2.84	6.77
12.5	6.14	0	3.67	0	3.82	0	2.82	6.80
14.5	6.76	0	3.54	0	3.76	0	2.80	7.30
16.5	6.50	0	3.50	0	3.65	0	2.80	7.83
18.5	6.12	0	3.49	0	--	--	2.78	8.06

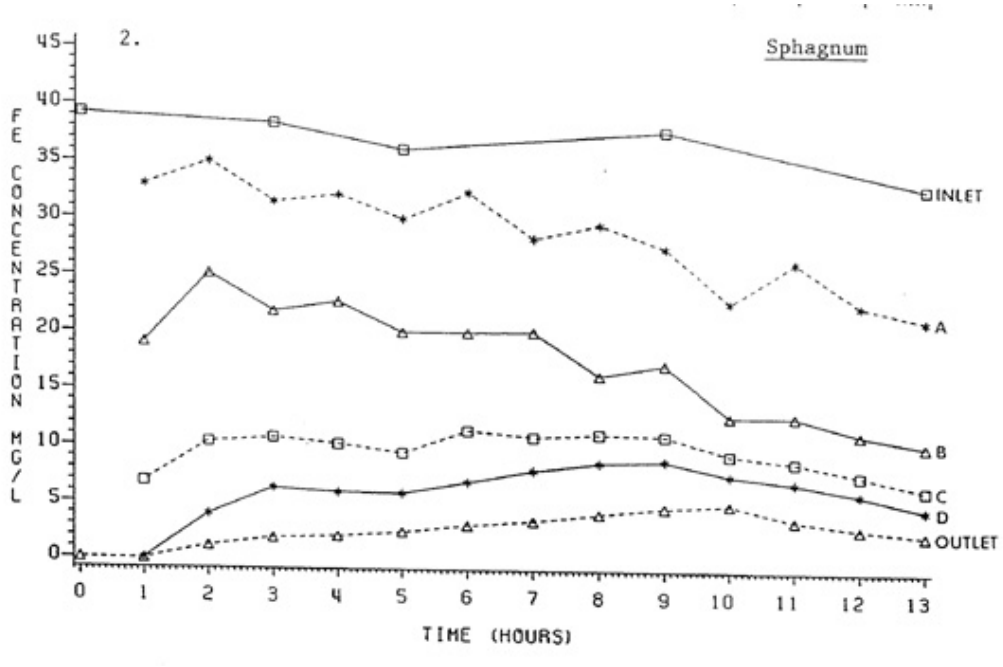
Table 2. pH at the outlet of Lanes 2, 3, and 4 during Experiment No. 2.

Hour	Lane 2 Drepanocladus	Lane 3 Sphagnum	Lane 4 Pohlia
initial	5.20	5.20	2.80
0	3.90	3.81	2.84
1	3.87	3.85	2.85
2	3.60	3.75	2.84
3	3.61	3.72	2.91
4	3.46	3.65	2.85
5	3.35	3.54	2.81
6	3.39	3.53	2.85
7	3.28	3.45	2.81
8	3.29	3.52	2.86
9	3.23	3.45	2.85
10	3.17	3.40	2.79
11	3.21	3.43	2.83
12	3.23	3.42	2.87
13	3.21	3.38	2.84

Table 2. pH at the outlet of Lanes 2, 3, and 4 during Experiment No. 2.

Hour	Lane 2 Drepanocladus	Lane 3 Sphagnum	Lane 4 Pohlia
initial	5.20	5.20	2.80
0	3.90	3.81	2.84
1	3.87	3.85	2.85
2	3.60	3.75	2.84
3	3.61	3.72	2.91
4	3.46	3.65	2.85
5	3.35	3.54	2.81
6	3.39	3.53	2.85
7	3.28	3.45	2.81
8	3.29	3.52	2.86
9	3.23	3.45	2.85
10	3.17	3.40	2.79
11	3.21	3.43	2.83
12	3.23	3.42	2.87
13	3.21	3.38	2.84





Figures 1-2. Fe concentrations at the inlet and outlet and four points (A, 2 ft; B, 4 ft; C, 8 ft; D, 12 ft) within Lanes 2 and 3 which contained Drepanocladus fluitans (1) and Sphagnu recuryum (2) during Experiment No. 2 in the simulated wetland.

Effectiveness of Fe removal changed more gradually along Lane 3 than Lane 2. After 13 h, 69% of the Fe had been removed at point B compared to a 92% reduction at the outlet. Burris et al. (1984) achieved an 87% reduction in Fe by S. recurvum. in an identical simulated wetland with an initial Fe concentration of 50 mg/l. The more effective removal of our system may result from the slower flow rate that we used (60 ml/min vs. 250 ml/min). Slow flow increases the contact time of the water with the vegetation thereby promoting cation exchange.

The Fe concentration in the outlet of Lane 4 (Pohlia) leveled off at 5-10 mg/l (Figure 3). The concentration of Fe was initially reduced to 5-6 mg/l as observed during Experiment No. 1. The explanations for this baseline level of Fe removal are not evident. As in Lanes 2 and 3, most of the Fe removal by the Pohlia occurred in the upper half of the lane. After 13 h, there was a 53% reduction by point C (lane middle) compared to 76% at the outlet. The large decrease in Fe concentration between points B and C in the lane may be due to differences in flow rate within the lane. The density of Pohlia was greatest in this segment. The water flow may have slowed considerably through this portion of the lane, improving the opportunity for metal removal by the Pohlia.

Tissue analyses

The concentrations of Fe in green tissues of Sphagnum. and Pohlia (Table 3) were comparable to levels reported in the same species from AMD sites (Webster 1985; McHerron 1986), but were higher than those from non-contaminated sites (Mayer and Gorham 1951). The Fe concentrations in the brown tissues from the simulated wetland were, however, approximately ten times higher than in the green tissues (Table 3). The water was in direct contact with the brown tissues so the Fe may be tightly bound on the plant surface or incorporated into the tissue. The Fe in the green portion was probably transported there by capillary action. Brown tissue of mosses is not necessarily dead, and is often capable of generating new shoots. It should be noted that in the Sphagnum. the brown coloration was not present when the plants were transplanted into the lane. The brown color is partially attributed to iron deposition.

The Pohlia did not have as high a concentration of Fe in the brown tissue as found in Oedogonium, Drepanocladus, and the brown portion of Sphagnum from the top of the lane. This may be a result of the lower effectiveness of the Pohlia in removing Fe from the water. However, the green Pohlia tissue had a higher Fe content than the green Sphagnum, possibly due to greater capillarity in the Pohlia.

All species, regardless of tissue type, showed a decline in Fe content along the length of each lane. In all lanes the concentration in the water was lower at the lower end (Table 1; Figures 1-3) so less Fe was available for uptake by the vegetation. in addition, the greatest drop in tissue Fe content in all species occurred in the upper half of the lane where the greatest Fe removal occurred. For example, Drepanocladus had only a 4 mg/l change in tissue content in the lower half (versus 7 mg/l in the upper half; Table 3) and had little change in Fe concentration in the water in the lower half during Experiment No. 2 (Figure 1). In contrast, Sphagnum showed a 70% decrease in water Fe concentration between points A and C at the end of Experiment No. 2 (versus a 48% reduction by Drepanocladus) and had a 33 mg/1 decrease in tissue Fe content between the same points. These data suggest that Sphagnum may be able to absorb a high amount of metal loading over a longer period of time before saturation occurs.

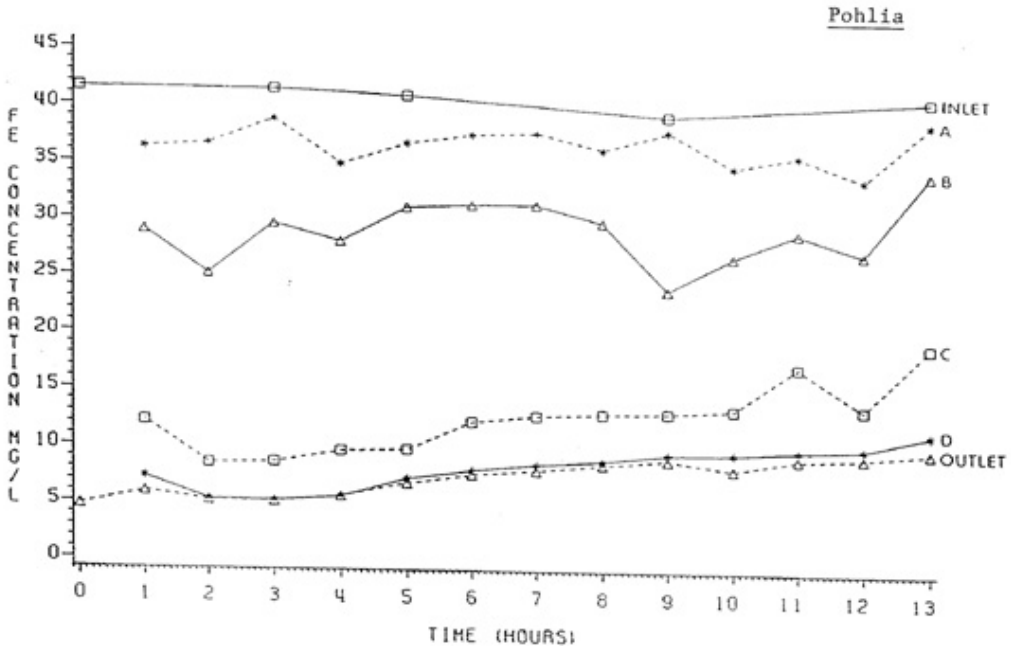


Figure 3. Fe concentrations at the inlet and outlet and four points (A, 2 ft; B, 4 ft; C, 8 ft; D, 12 ft) within Lane 4 containing Pohlia nutans during Experiment No. 2 in the simulated wetland.

Table 3. Fe concentrations (mg/g) in the tissues of four plant species following exposure to Fe in the simulated wetland.

		Location along lane		
Species	Tissue type	upper	middle	lower
Oedogonium sp.	composite	--*	34.34	31.88
Drepanocladus fluitans	composite	44.28	36.56	33.22
Sphagnum recurvum	green	1.90	0.55	0.30
	brown	40.89	8.36	1.53
Pohlia nutans	green	14.61	2.47	1.45
	brown	33.75	28.84	21.69

*Insufficient tissue was available to be analyzed.

Table 3. Fe concentrations (mg/g) in the tissues of four plant species following exposure to Fe in the simulated wetland.

Species	Tissue type	Location along lane: upper	middle	lower
Oedogonium sp.	composite	--*	34.34	31.88
Drepanocladus fluitans	composite	44.28	36.56	33.22
Sphagnum recurvum	green	1.90	0.55	0.30
	brown	40.89	8.36	1.53
Pohlia nutans	green	14.61	2.47	1.45
	brown	33.75	28.84	21.69

*Insufficient tissue was available to be analyzed.

Microbial Populations

Several researchers recently reported finding significant numbers of heterotrophs in mine drainage (e.g., Wichlacz 1980; McHerron 1986). The total numbers of heterotrophs found in the simulated wetland (Table 4A) are comparable to those found in Sphagnum recurvum in the same simulator (Gerber et al. 1985). These numbers are higher than those typically found in other types of stressful environments. There was no apparent difference in total counts between the four plant species tested. However, there tended to be fewer bacteria on the low organic and medium protein media. Since the microbes in the simulated wetland were epiphytic, and thus growing in a high nutrient environment, these lower counts would be expected. one would expect to find higher numbers of heterotrophs on the brown portions of the mosses because more substrate is available due to tissue degradation. However this pattern was not observed in the Sphagnum and Pohlia.

Fe-oxidizing microbes were associated with each plant species (Table 4A), and consisted mostly of fungi. Although Oedogonium had the lowest total counts, a higher proportion of the colonies were oxidizers. Sphagnum and Pohlia had the lowest proportion of Fe oxidizers, but had higher total counts. Brown plant material had a higher proportion of oxidizers than green tissue. There was no apparent correlation between bacterial counts and the effectiveness of the species in removing Fe.

As is typical of a stressful environment, the number of recognizably distinct colony types, an indicator of species diversity, was low, but was similar to levels found in AMD wetlands. Species diversity also did not differ significantly between plant species (Table 4B). There was no difference in bacterial diversity between brown and green tissues. Additional microbial counts from wetlands unimpacted by AMD are needed.

SUMMARY

The experiments in the simulated wetland indicated that all four plant species could improve the water quality by removing Fe. Sphagnum recurvum was the most effective, followed by Drepanocladus fluitans and Pohlia nutans. Oedogonium sp. appeared to remove Fe as well, however, full evaluation of its effectiveness was not possible. Sphagnum and Drepanocladus lowered the pH of the water, whereas Pohlia did not. These findings are consistent with a cation-exchange mechanism.

All mosses were able to remove at least 50% of the Fe in the upper half of the lane, and showed little or no signs of becoming saturated after the three months of Fe exposure. An analysis of the Fe content in the tissues indicates that Sphagnum has the greatest capacity to remove Fe. The highest Fe content was

found in the upper half of each lane, where concentrations of Fe in the water were highest. Brown tissue, in direct contact with the water, had a higher Fe content than green tissue. However, the brown tissue of Sphagnum in the lower part of the lane had the same amount of Fe as the green tissue in the upper part of the lane. This indicates that Sphagnum can withstand extended Fe loading before saturation occurs.

Both heterotrophic and oxidizing bacteria may remove Fe from AMD. In the simulated wetland, there was no obvious association between effectiveness of metal removal and microbial populations. Fe oxidizers were found in all lanes. Bacteria which reduce Fe may also be present, but these were not cultured. The largest population of heterotrophs were those which could grow in a high organic environment. Total numbers of bacteria were higher than, but species diversity similar to what one would expect to find in a stressful environment.

Table 4. Total numbers of heterotrophic bacteria (mean CFU/g fresh wt. ± SD) (A) and numbers of recognizably distinct colony types (B) on 6 different media from 4 lanes of the simulated wetland.

	Genus	Sample type	Fe-rich medium	% oxidizers*	low organics	medium organics	high organics	medium protein	high protein
A.	Oedogonium	composite	2.2×10^4 $\pm 1.2 \times 10^4$ n=3	89.0±19.0 n=3	7.5×10^4 $\pm 3.1 \times 10^4$ n=2	3.6×10^7 $\pm 3.5 \times 10^7$ n=3	1.5×10^8 $\pm 1.0 \times 10^8$ n=2	2.2×10^5 $\pm 2.5 \times 10^5$ n=3	6.3×10^6 n=1
	Drepanocladus	composite	1.5×10^5 $\pm 0.9 \times 10^5$ n=3	28.2±14.5 n=3	8.9×10^4 $\pm 4.3 \times 10^4$ n=2	8.9×10^6 $\pm 5.3 \times 10^6$ n=3	2.1×10^7 $\pm 1.3 \times 10^7$ n=3	3.2×10^4 $\pm 2.5 \times 10^4$ n=3	1.4×10^6 n=1
	Sphagnum	green	9.6×10^4 $\pm 1.2 \times 10^5$ n=3	11.8±12.6 n=3	1.1×10^4 $\pm 0.9 \times 10^4$ n=2	2.2×10^6 $\pm 0.9 \times 10^6$ n=3	3.3×10^6 $\pm 1.2 \times 10^6$ n=3	2.2×10^4 $\pm 1.9 \times 10^4$ n=3	1.6×10^5 $\pm 0.4 \times 10^5$ n=2
		brown	5.7×10^4 $\pm 1.8 \times 10^4$ n=3	37.5±17.7 n=2	7.0×10^4 n=1	4.2×10^6 $\pm 3.0 \times 10^6$ n=3	1.4×10^7 $\pm 1.3 \times 10^7$ n=3	2.6×10^4 $\pm 0.5 \times 10^4$ n=3	7.3×10^4 $\pm 2.8 \times 10^4$ n=2
	Pohlia	green	2.4×10^6 $\pm 1.6 \times 10^6$ n=3	6.0± 4.5 n=2	6.4×10^4 n=1	2.4×10^7 $\pm 1.0 \times 10^7$ n=3	4.2×10^7 n=1	2.8×10^4 $\pm 4.0 \times 10^4$ n=3	1.0×10^6 n=1
		brown	5.8×10^5 $\pm 3.9 \times 10^5$ n=3	48.0 n=1	5.2×10^4 $\pm 6.8 \times 10^4$ n=2	2.0×10^6 $\pm 2.1 \times 10^6$ n=3	7.6×10^6 $\pm 7.8 \times 10^6$ n=3	5.6×10^4 $\pm 4.1 \times 10^4$ n=3	6.4×10^4 $\pm 1.3 \times 10^4$ n=2
B.	Oedogonium	composite	2.0± 1.0 n=3		9.0± 0 n=2	8.0± 1.0 n=3	12.5± 5.0 n=2	11.0± 1.0 n=3	4.7± 1.1 n=3
	Drepanocladus	composite	6.7± 2.1 n=3		8.5± 3.5 n=2	7.0± 2.0 n=3	18.3± 8.0 n=3	11.0± 4.6 n=3	4.0± 0 n=2
	Sphagnum	green	4.3± 1.5 n=3		6.0± 0 n=2	4.7± 1.5 n=3	5.5± 5.0 n=2	4.7± 1.1 n=3	1.7± 0.6 n=3
		brown	4.0± 2.0 n=3		6.0 n=1	6.0± 1.0 n=3	11.0± 4.6 n=3	4.7± 0.6 n=3	1.5± 0.5 n=2
	Pohlia	green	9.7± 2.1 n=3		9.0 n=1	7.0± 1.7 n=3	12.0 n=1	7.0± 0 n=3	2.3± 0.6 n=3
		brown	5.7± 2.1 n=3		8.0± 2.0 n=2	5.0± 1.7 n=3	13.3± 5.5 n=3	4.3± 1.1 n=3	2.7± 0.6 n=3

*Percent of heterotrophs on Fe-rich medium which oxidized Fe.

This research was supported by grants-in-aid from Peabody Coal Company and Pennsylvania Power and Light Company, and a Ben Franklin Grant from the Advanced Technology Center of Central and Northern

Pennsylvania, Inc. The assistance of D. Gerber and T. Omeis is gratefully acknowledged.

REFERENCES

Burris, J.E., D.W. Gerber, and L.E. McHerron. 1984. Removal of iron and manganese from water Sphagnum moss. pp. 1-13. IN J.E. Burris (ed.), Treatment of Mine Drainage by Wetlands. Contribution no. 264 Dept. Biol. The Pennsylvania State University, University Park, PA.

Gerber, D.W., J.E. Burris, and R.W. Stone. 1985. Removal of dissolved iron and manganese ions by a Sphagnum. moss system. pp. 365-372. IN R.P. Brooks, D.E. Samuel, and J.B. Hill (eds.), Wetlands and Water Management on Mined Lands. Proceedings of a Conference. The Pennsylvania State University, University Park, PA.

Mayer, A.M. and E. Gorham. 1951. The iron and manganese contents of plants present in the natural vegetation of the English Lake District. Ann. Bot. N. S. 15: 247-263.

McHerron, L.E. 1986. Removal of Iron and Manganese from Mine Drainage by a Wetland: Seasonal Effects. M.S thesis. The Pennsylvania State University, University Park, PA.

Webster, H.J. 1985. Elemental analyses of Pohlia nutans growing on coal seeps in Pennsylvania. J. Hattori Bot. Lab. 58: 207-224.

Wichlacz, P.L. 1980. Acidophilic Heterotrophic Bacteria from Acid Mine Drainages in Central Pennsylvania. M.S. thesis. The Pennsylvania State University, University Park, PA.

Wieder, R.K. and G.E. Lang. 1982. Modification of acid mine drainage in a freshwater wetland. pp. 43-53. IN B.R. McDonald (ed.), Proceedings of the Symposium on Wetlands of the Unglaciated Appalachian Region. West Virginia University, Morgantown, WV.

Stone, R.W. 1984. The presence of iron- and manganese-oxidizing bacteria in natural and simulated bogs. pp. 30-36. IN J.E. Burris (ed.), Treatment of Mine Drainage by Wetlands. Contribution no. 264 Dept. Biol. The Pennsylvania State University, University Park, PA.