THE SIMCO #4 WETLAND: BIOLOGICAL PATTERNS AND PERFORMANCE OF A WETLAND RECEIVING MINE DRAINAGE. I[1]

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Abstract.--In 1985 a 3,000-m^2, three-celled wetland was installed at the Peabody Coal-American Electric Power Simco #4 deep coal mine site near Coshocton, OH. The wetland included a limestone/compost substrate planted with *Typha latifolia* L. (cattail). The deep mine seepage pH is near 6.0, total iron 80-241 mg/L, and acidity 15-389 mg/L. The percent reduction in iron concentration in effluent water relative to influent water of the wetland significantly exceeded pre-wetland percent reduction, improving from 28% during the first winter after construction to 62% in the summer of 1987. Further site modifications in 1987 elevated the percent iron reduction of the wetland to 70-85%. Iron reduction corresponds to greater wetland area, higher cattail density and increased plant coverage. Prominent plants in the wetland besides cattail are *Leersia oryzoides* (L.) Sw. (cutgrass) and several algal species. Cattail and cutgrass coverage increased from 1986 to 1987. Roots of cattail accumulated up to 5 times the iron content of roots from a control site.

INTRODUCTION

In the last 10 years over 100 wetlands have been constructed in the coal-bearing regions of Ohio, Pennsylvania, Maryland, and West Virginia in attempts to treat acidic mine water (Kleinmann and Girts 1987). The alternative can be costly: chemical treatment in settling ponds may cost a mining company as much as $50,000/year. Usually acidic mine water exceeds Federal regulations for iron (3 mg/L), manganese (2 mg/L), pH (6-9), or a combination of these. Sulfates and acidity can also be high. Wetlands have shown promise in lowering levels of iron and manganese (Wieder et al. 1982; Brooks et al 1985). Monitoring the performance and composition of some of these wetlands will return useful knowledge on their potential.

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WETLAND CONSTRUCTION AND MODIFICATIONS

The Simco #4 underground mine began operation in 1970 and ceased operation on October 20, 1978. The location of the portal was a pre-existing strip bench operation of 1961. This mine operated 3 conventional sections mining the Kittanning coal seam. Run-of-mine coal was delivered to the Columbus & Southern Ohio Electric Conesville Generating Station.

Upon abandonment the mine was left unsealed and the highwall above the portal was not reclaimed to approximate original contour. In May 1979 the Division of Mines and the Mine Safety and Health Administration approved plans for sealing the Simco #4 underground mine. The drift openings were sealed with 24 in. concrete or concrete bulkhead constructed with pilasters. One pilaster was used for widths under 16 ft. and two pilasters were used for widths greater than 16 ft. The seals were built into the floor, ribs, and top, and the tops were supported to make the areas safe prior to construction. All three openings at the Mine #4 portal were sealed using this procedure, and the pre-existing highwall was subsequently backfilled.

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In 1980, water treatment began for discharge seepage that developed from the mine near the base of the backfill. Discharge specifications were met by treating with soda ash briquettes. During the next several years, the flow increased to approximately 120 gpm while the water quality remained poor during the period 1980 through 1985: pH, 5.7-6.3; total iron, 145-241 mg/L; total manganese, 3.1-6.0 mg/L; and acidity, 140-389 mg/L.

As the flow increased it became increasingly difficult to treat the discharge effectively with soda asd briquettes. In an attempt to improve the treatment system and lower treatment costs, Simco/Peabody decided to use caustic soda and install an aeration system to elevate pH and enhance the settling of iron and manganese.

From March 1980 through June 1985 Simco/Peabody studied several options to effectively seal the mine to eliminate the discharge. One such method proposed sealing the Simco #4 underground mine with fixed Flu Gas Desulfurization Sludge from the Columbus & Southern Ohio Electric Conesville Generating Station. However, both the Office of Surface Mining Reclamation and Enforcement and the Ohio Environmental Protection Agency raised numerous questions and informational requests on the use of scrubber sludge. Concern was expressed about the long-term liability of the sludge if it were to become hazardous by decree or demonstration and also over the sludge fixation agent.

It was thought that the proposed innovative sealing scheme may elicit a response from the regulatory agencies relieving liability and setting up a cooperative research effort; however, this did not happen. Since the Simco #4 underground mine was under Notice of Violation (NOV) for its discharge, Peabody Coal developed cost estimates for the double bulkhead seal with center plug (the more conventional approach).

Complicating the water discharge problem was an NOV issued by the Ohio Department of Natural Resources (ODNR), Division of Reclamation. The NOV issued May 1983 required Simco/Peabody to remove all treatment equipment, ponds, and totally reclaim the area. Otherwise, final bond release could not be obtained and the permit could be revoked. Abatement extensions were granted by ODNR while Peabody did further development on sealing methods. While the plans to reactivate the entries and construct hydraulic seals within the mine were developed and final construction details and bid requests were prepared, it was decided that such sealing was too costly, time consuming, and would not provide any assurance of complete effectiveness.

Therefore, in April 1985, Peabody Coal contacted a wetland consultant, B. Pesavento, to perform a site evaluation of the abandoned Simco #4 underground mine. His analysis indicated that a wetland could be constructed that would sufficiently improve the discharge, bringing it within applicable discharge limits without the use of the existing treatment equipment. A proposal based upon Pesavento's recommendation was submitted to the ODNR in July 1985. After careful review the ODNR permitted the installation of the wetland as a possible alternative to hydraulic sealing of the mine.

Site conditions at the mine were conducive to construction of a wetland treatment system. The abandoned strip pit west of the backfilled portal area through which the discharge (120 gpm) passed provided more than adequate area and sufficiently low slopes for construction of a wetland system. The pit floor was graded and a layer of crushed limestone (6 inches thick) was applied in the initial stages of wetland construction. The crushed limestone was covered with an organic-rich, deep-rooting medium (18 inches) in which lime was incorporated. _Typha_ rhizomes were planted with a density of $3\text{-}4/m^2$.

The wetland system was constructed in segments (cells) to provide proper gradients to restrict flow velocities and to promote uniform water dispersal throughout the wetland. Segmenting the wetland provided a gradual introduction of the discharge water into the wetland, allowing the vegetation to establish prior to the introduction of the entire discharge.

Construction of the wetland was started on October 14, 1985 and completed 20 November 1985. Three wetland cells separated by mixing pools were established (fig. 1). The total wetland area is about 3,000 m^2. Water quality, vegetation, and aerobic microbial populations have been monitored since June 1986, and are reported herein.

Periodically the wetland has been limed and/or fertilized with phosphate (table 1) in an attempt to increase iron removal and promote the growth of algae. Such applications have now stopped. Despite the liming, pH was unaffected, and the iron removal efficiency of the wetland did not change significantly. However, the iron removal efficiency of the ditch below the wetland was distinctly elevated in the summer of 1986, coinciding with the largest application of lime. Phosphate applications were relatively small.

During the second winter after installation (1986-7) the water below the wetland at station DNO2 required chemical treatment to remove more iron. At the time the water level and flow rate was judged to be too high in Cells 1 and 2, and the use of diversion ditches was recommended to effectively cut flow rate in half and lower the water depth. In addition, a series of hay bale dikes was recommended to be installed as baffles to increase retention time and promote bacterial activity. These changes were completed on September 1, 1987 (fig. 2). By this time the wetland had improved enough in efficiency to discontinue chemical treatment. However, recent analyses show the wetland to be performing at its highest efficiency, with respect to iron removal, since installation.

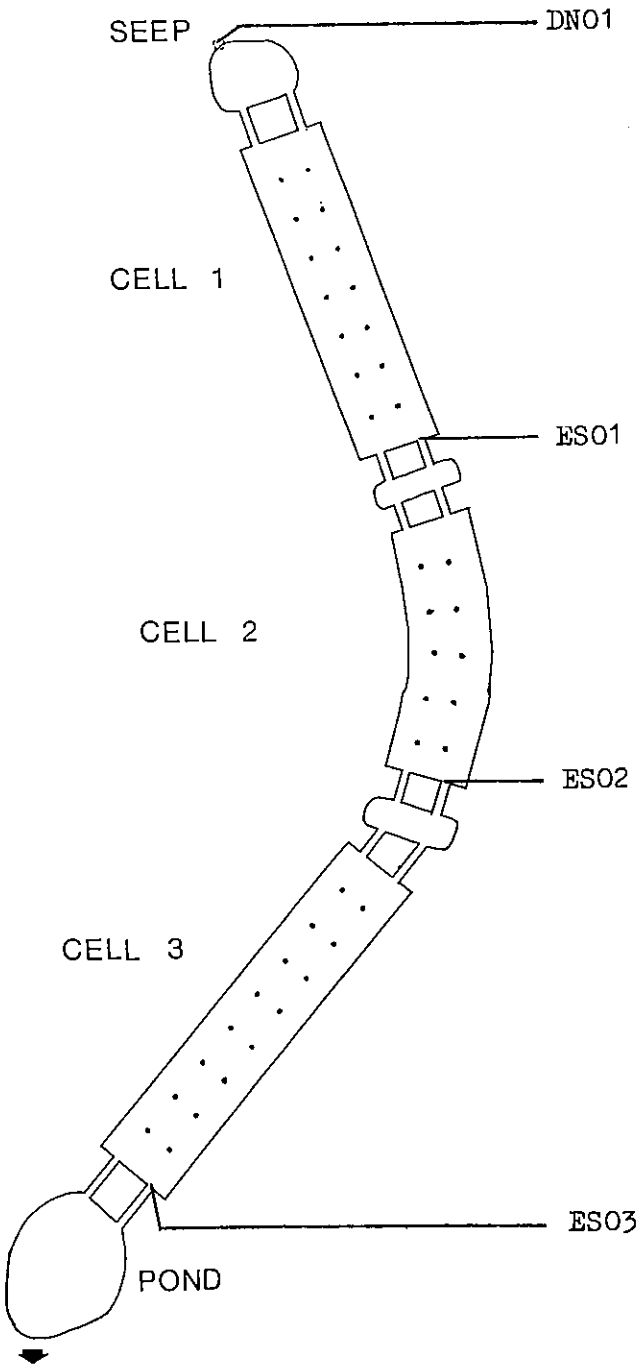

Figure 1.--Diagram of Simco #4 Wetland showing water sampling points at right. Dots represent permanent quadrat stations. Water sampling point DNO2, not shown, is approximately 100-m below pond outlet. Cell areas: Cell 1: 1,008 m^2; Cell 2: 864 m^2; Cell 3: 1,200 m^2.

Table 1.--Dates and amounts of agricultural lime and phosphate (di-ammonium phosphate:0-45-0) applied to the wetland.

Date	Lime (lbs)	Fertilizer (lbs)
Dec 1985	--	200
Feb 1986	--	200
Mar 1986	--	200
Jul 1986	8,000	--
Nov 1986	950	200
Dec 1986	1,000	200
Jan 1987	--	150
Apr 1987	--	150
Jul 1987	--	150
Sep 1987	--	150

METHODS

Water Chemistry

Water was collected by Peabody Coal at 2-3 week intervals at the seep, the outfall of each of the three wetland cells, and 100 m downstream of the wetland, just prior to entering the treatment pond. Water was analyzed by the Peabody Coal Lab for acidity, total iron, total manganese, and sulfate. In the field, pH, temperature, and the water flow rate discharging from the wetland were taken. Acidity was determined using an Orion 407/A pH meter by titration (APHA 1980). Metal concentrations were determined with a Perkin/Elmer 4000 and 5000 atomic absorption spectrophotometer using standard techniques. Sulfates were determined turbidimetrically using a Hach Turbidimeter Model 2100A (through July 1987) and a Hach Ration Turbidimeter Model 18900 (August 1987 on).

On a quarterly basis The Pennsylvania State University (PSU) collected water samples at the permanent sampling stations established within the wetland (fig. 1). These were then filtered (0.45 µm) and acidified (HNO_3) in the field and analyzed by atomic absorption spectrophotometry using a Buck 200 AA for determination of dissolved iron and manganese.

Vegetation

Vegetation was surveyed by PSU at each of the 40 permanent sampling stations on a quarterly basis beginning in July 1986. Canopy coverage was estimated (Daubenmire 1970) in the summers of 1986 and 1987, the first two growing seasons for the wetland. In 1987 algal presence (January) and cover (April) in each quadrat were

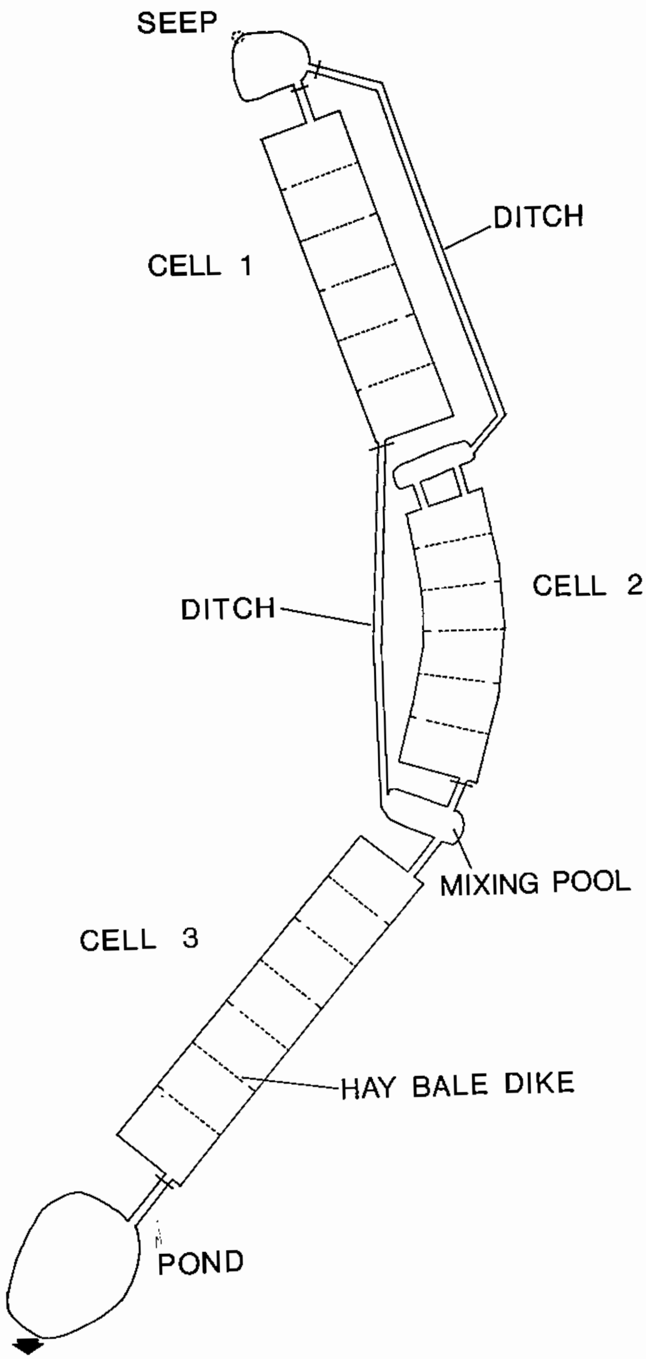

Figure 2.--Diagram of Simco #4 Wetland after site modifications of August 31, 1987. Hay bale dikes were spaced at approximately 35-ft. intervals.

recorded. In addition, the number of cattail shoots was counted in the quadrats in April and August 1987.

Metals in Typha Structures

Whole cattail plants were collected in April and August 1987 and returned to the lab in plastic bags. These plants were then separated into leaf blades; leaf bases; inner, outer and entire rhizomes; and roots. Damaged or dead plant parts and visible contaminants were removed, followed by cleaning in tap water, with vigorous rinses and gentle brushing to remove dislodgeable surface contaminants. Samples were then oven-dried at 70°C, ground in a Wiley mill, and stored in sealed glass jars. Wet acid digestion was used, modified from the procedures in Allen et al. (1986). The dried plant material (500 mg) was acid digested at 160°C, first with 10 mL dH_2O (distilled water) and 5 mL concentrated H_2SO_4 for 20 minutes, then 5 mL concentrated HNO_3 was added and digestion continued for 10 minutes. After cooling, samples were filtered (Whatman GFC glass filters), diluted to a volume of 100 mL using deionized distilled H_2O, and stored at 2°C. Two aliquots of each digestate were analyzed by atomic absorption spectrophotometry for iron and manganese concentrations. Each value reported represents an average of the two aliquots. Control plants were collected from a non-impacted site near DuBois, PA and analyzed as above.

Microbial Studies

The microbiological work has essentially been an attempt to discover the major nutritional types of microorganisms present in the wetland, their total number, and their diversity. Until recently heterotrophic microorganisms were not thought to occur in high iron-loaded waters. However, Wichlacz (1980) showed that acidophilic heterotrophs were present in acid mine waters, a result confirmed by McHerron (1986).

Samples were collected in September 1986 and in January, April, and October 1987 at 20 locations along the length of the wetland. Depending on the nature of the sediments, samples were obtained in one of two ways. If the sediments were solid enough to hold a shape, solid cores were taken with a 3-cm-diameter metal cylinder. If the sediments were loose and watery, samples were collected by holding a sterile test tube just below the surface of the sediment, in front of the flow. When the tube was uncapped, the sediments flowed into the tube with as little disturbance as possible. The samples were kept on ice during transportation to the laboratory.

For solid core samples, the outer portion of the core was removed with a sterile razor blade to expose the inner, presumably undisturbed, portion. A subsample of 2.5 g was blended in a Waring blender with 1% sodium pyrophosphate (Balkwill 1977). The purpose of this step was to release the microorganisms from the soil particles; sodium pyrophosphate aids in breaking up the soil aggregates. If the test tube samples were watery, the sediments were blended with enough sodium pyrophosphate to give an approximate 1% solution. From this point on, both types of samples were treated in the same manner.

The suspensions were immediately diluted using sterile distilled in a series of 1:00; 1:1,000; 1:10,000; and 1:100,000. Each dilution tube was placed on a vortexing machine before any liquid was drawn from it so the particles did not have time to settle out. All transfers were made with micropipettes. Each dilution was then plated out in triplicate on seven different kinds of agar media. The media used ranged from very simple and defined (agar water and inorganic media) to complex and undefined (PTYG and BHI), in order to select for a gradient of nutritional types (Balkwill and Ghiorse 1985). Both iron-supplemented and manganese-supplemented inorganic media were used to select for iron and manganese oxidizers. Plates were surface inoculated and incubated at 27°C.

Colonies were counted and described for plates on which 30-300 colonies developed. The counts included fungi, actinomycetes, and bacteria. The final counts and descriptions were made at 15-30 days, depending on the type of medium used. A series of counts was also made within this time period to estimate growth rates (data not presented). Colony types were distinguished on the basis of relative size, color, conformation, border, and elevation. The presence of iron or manganese oxidizers was determined visually by the formation of a deep blue color after flooding the colonies with 0.2% tetramethyl benzidine in 2M acetic acid (Stone 1984). The iron and manganese oxidizers were isolated from these plates for further study. Gram stains were performed on the predominant colony types, and cellular morphology was described.

RESULTS

Water Chemistry

Flow. Flow rate measured at the outflow of the wetland ranged from 100-160 gpm from September 1986 to August 1987, but declined to 50-65 gpm in September 1987. Lower than normal precipitation in 1987 in combination with the site modifications of late August 1987 may have contributed to this flow reduction.

pH. Prior to wetland installation the pH on average decreased from 6.16 to 5.88 between the seep (DNO1) and the final sample point (DNO2; table 2). Following wetland installation the pH has remained relatively constant. Although too early to detect a pattern, following site modifications of August 1987 the pH of water leaving Cell 3 was the lowest (5.99) since wetland installation.

Iron. Total iron at the seep ranged from 80-241 mg/L from 1983-1987, but was usually 100-120 mg/L (table 3). Pre- and post-wetland iron levels can be compared at the end of the 100 m-long ditch below the wetland (station DNO2, approximately 100-m below the pond outlet) which was sampled prior to and after wetland installation. "Efficiency" (or more properly the percent reduction in iron concentration of effluent water relative to influent water) in removing iron is defined as:

$$\frac{\text{Total Fe conc. at DNO1} - \text{Total Fe conc. at DNO2}}{\text{Total Fe conc. at DNO1}}$$

Table 2.--Average pH values by season of each wetland cell and ditch outfall; N=5 for pre-wetland means, N=4-7 for post-wetland means.

Location	Pre-wetland (1983-1985)	Winter 1985-6	Spring 1986	Summer 1986
Seep	6.16	6.45	6.40	6.40
Cell 1	--	6.07	6.52	6.40
Cell 2	--	5.92	6.46	6.22
Cell 3	--	6.03	6.40	6.22
Ditch End	5.88	6.07	6.50	6.24

	Fall 1986	Winter 1986-7	Spring 1987	Summer 1987	Fall 1987*
Seep	6.46	6.34	6.25	6.36	6.39
Cell 1	6.16	6.48	6.45	6.33	6.29
Cell 2	6.16	6.48	6.40	6.31	6.27
Cell 3	6.47	6.36	6.27	6.19	5.99
Ditch End	6.16	6.50	6.43	6.26	6.18

* after site modifications of August 31, 1987

Winter = Dec-Feb
Spring = Mar-May
Summer = Jun-Aug
Fall = Sep-Nov

Prior to wetland installation the total iron concentration was reduced by an average of 32% between DNO1 and DNO2 (180-122/180). During the first winter following installation the iron reduction efficiency for the wetland (at DNO2) was 35% (table 4). This efficiency rose through the spring and summer to 81% in July 1986, averaging 75% in its first summer and 70% in the second summer. The efficiency in the second winter (1986-7) exceeded that of the first winter, 53% to 35%, as did the efficiency of the second spring to the first, 62% to 47%. Using this efficiency index, this wetland is improving over time (fig. 3).

Because station DNO2 is 100-m below the wetland, a more accurate gauging point for wetland efficiency is ES03, which is at the outfall of the third (final) wetland cell. The efficiency at ES03 reflects the effect of the wetland system proper on water quality. The cumulative percent reduction at ES03 (table 4) climbed steadily from 28% to 56% in the summer of 1986 and remained near that level until the following summer, when it rose to 62%. Thus, (a) there was not a significant dropoff in the wetland's efficiency in the reduction of iron over the winter, and (b) the level of iron

Table 3.--Average total iron (mg/L) by season of each wetland cell and ditch outfall; N=5 for pre-wetland means, N=4-7 for post-wetland means.

Location	Pre-Wetland (1983-1985)	Winter 1985-6	Spring 1986	Summer 1986
Seep	180 (range:144-241)	116	106	127
Cell 1	---	99	93	98
Cell 2	---	93	81	77
Cell 3	---	84	65	56
Ditch End	122	75	55	32

	Fall 1986	Winter 1986-7	Spring 1987	Summer 1987	Fall 1987*
Seep	144	113	100	87	100
Cell 1	123	100	78	68	62
Cell 2	105	86	63	59	59
Cell 3	74	60	42	34	26
Ditch End	58	52	38	26	19

* after site modifications of August 31, 1987

Winter = Dec-Feb
Spring = Mar-May
Summer = Jun-Aug
Fall = Sep-Nov

Table 4.--Average percent reductions in total iron concentration by season of each wetland cell, the ditch, cumulative (cum.) for the wetland proper, and end of ditch; N=4-7 for each season.

Component	Winter 1985-6	Spring 1986	Summer 1986	Fall 1986
Cell 1	14	12	22	17
Cell 2	6	12	20	14
Cell 3	12	18	27	29
Ditch	10	16	42	22
Cum. wetland	28	37	56	53
Cum., end of ditch	35	47	75	59

Component	Winter 1986-7	Spring 1987	Summer 1987	Fall 1987*
Cell 1	17	21	22	38
Cell 2	15	17	17	41
Cell 3	30	33	42	56
Ditch	8	13	22	26
Cum. wetland	51	55	62	73
Cum., end of ditch	53	62	70	81

* after site modifications of August 31, 1987

Winter = Dec-Feb
Spring = Mar-May
Summer = Jun-Aug
Fall = Sep-Nov

reduction was much greater in the second winter (51%) than the first winter (28%). In the interval between the first and second winters this constructed wetland experienced its first growing season.

A knowledge of the efficiency of each wetland cell is valuable in further evaluating the wetland system. The percent reduction of Cell 1 is the efficiency up to ES01; the percent reduction of Cell 2 uses ES02 and ES01 as reference points; and the percent reduction of Cell 3 uses ES03 and ES02 as reference points. Prior to the site modifications, Cells 1 and 2 showed peak efficiencies in the summers of 1986 and 1987. The variation here is high, but 3-month summer averages for Cells 1 and 2 are 22% and 20%, respectively (table 4). The efficiencies for these two cells decreased slightly after the summer (3-month averages of 17% and 14%). Cell 3 efficiency, however, has increased steadily since installation, performing at twice the efficiency of Cell 2. The improvement of the wetland system proper from 28% (1986) to 51% (1987) in winter efficiency is in large part due to the high winter efficiency of Cell 3. The decline in cumulative efficiency at point DN02 in the fall and winter of 1986-7 is therefore not due to the action of the wetland itself, but to the decreased removal in the ditch between ES03 and DN02. Iron removal by the ditch ranged from a high of 42% in summer 1986 to only 8% in winter 1986-7 (table 4).

Water samples were taken within the wetland at each of 40 stations on a quarterly basis. Representative results for August 1987 (fig. 4) indicate that no sharp transition zones of iron reduction exist; a gradual decrease in iron content in the water occurs from inlet to outlet. Higher iron levels are present on the right-hand (highwall) side of the wetland; this is probably due in large part to the much greater flow and channeling observed on this side prior to the August 31, 1987 modifications.

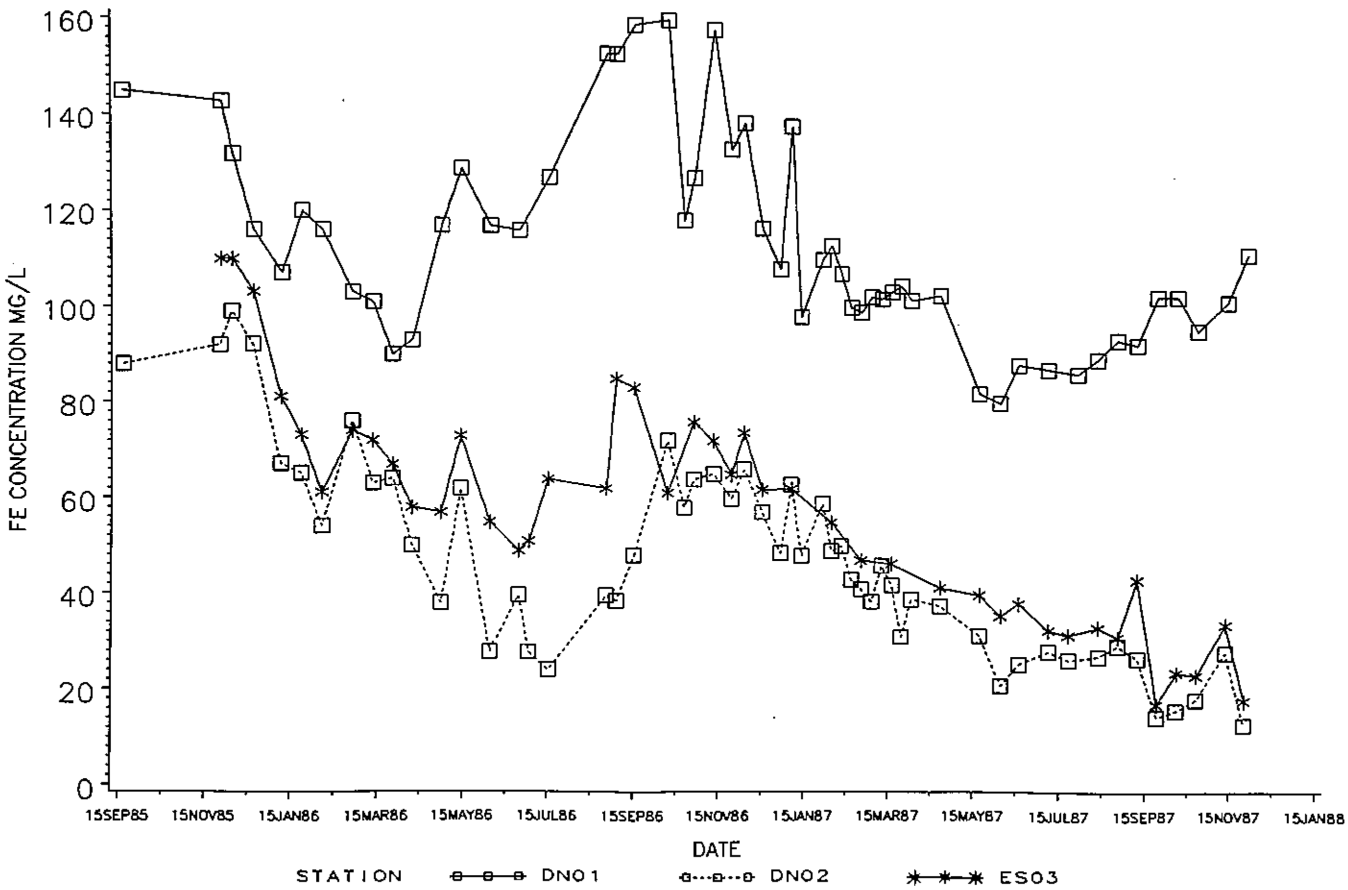

Figure 3.--Total iron concentrations at DN01 (seep), ES03 (wetland proper outlet), and DN02 (100-m below wetland).

The introduction of hay bale dikes and the diversion of half the water around each of Cells 1 and 2 (performed between the summer and fall of 1987) resulted in a further reduction of iron in the water. Although we cannot predict how the wetland would have performed without modification, we can compare patterns between the summer-fall of 1986 and the summer-fall of 1987. In the fall of 1986 the cumulative percent reduction in the iron concentration after passing through the wetland was 53% (table 4), 3% lower than the summer 1986 efficiency. However, the iron reduction efficiency in the fall of 1987 was 73%, 11% higher than that of the preceding summer. Each wetland cell increased in efficiency (a direct increase of 14% to 24%; table 4), with the middle cell showing the most dramatic improvement. Prior to site modifications, summer had been the season of peak performance for iron reduction (56% and 62%). However, in the fall of 1987 after modification, an all-time seasonal high of 73% was recorded (table 4).

Manganese. Total manganese in the seep water ranged from 1.66-6.02 mg/L from 1983-1987, averaging 2.12 since 1985 (N=43). Wetland outlet water over the same period has ranged from 1.81-6.09 mg/L, averaging 2.28 mg/L (N=43). Thus, the Mn concentration in the water increased by 8% between seep and wetland outlet, with the elevation occurring in Cells 1 and 2. Although the manganese levels are a minor concern due to their low levels, the probable explanation of the slight elevation in Mn over the first two wetland cells was found to be a small surface side seep of <1 gpm alongside Cell 1 that contained about 5 mg/L Mn and negligible Fe.

Acidity. Pre-wetland acidity at the seep and the ditch outfall averaged 207 and 187 mg/L, respectively: an improvement of 10%. The effect of the wetland was to reduce acidity (table 5): from the summer of 1986 forward the wetland has lowered acidity by at least 63%. Cell 3 has been the most effective cell in reducing acidity in five of the seven seasons, followed by Cell 1.

Sulfate. No data on sulfates are available prior to wetland installation. Since late 1985, sulfates in the seep water have averaged 1,226 mg/L (N = 40; range = 964-1,841 mg/L). The wetland has lowered this level by 7%, (N = 40; range = 425-1,931 mg/L).

Vegetation

Following the initial Typha latifolia transplants the wetland has basically been allowed to establish on its own. Seventeen species of plants have appeared in the wetland, but only a few account for a significant biomass based on coverage (table 6): Typha latifolia, Leersia oryzoides, and mixed algal populations including primarily Oscillatoria sp., Chlamydomonas polypyrenoideum, and Euglena sp.

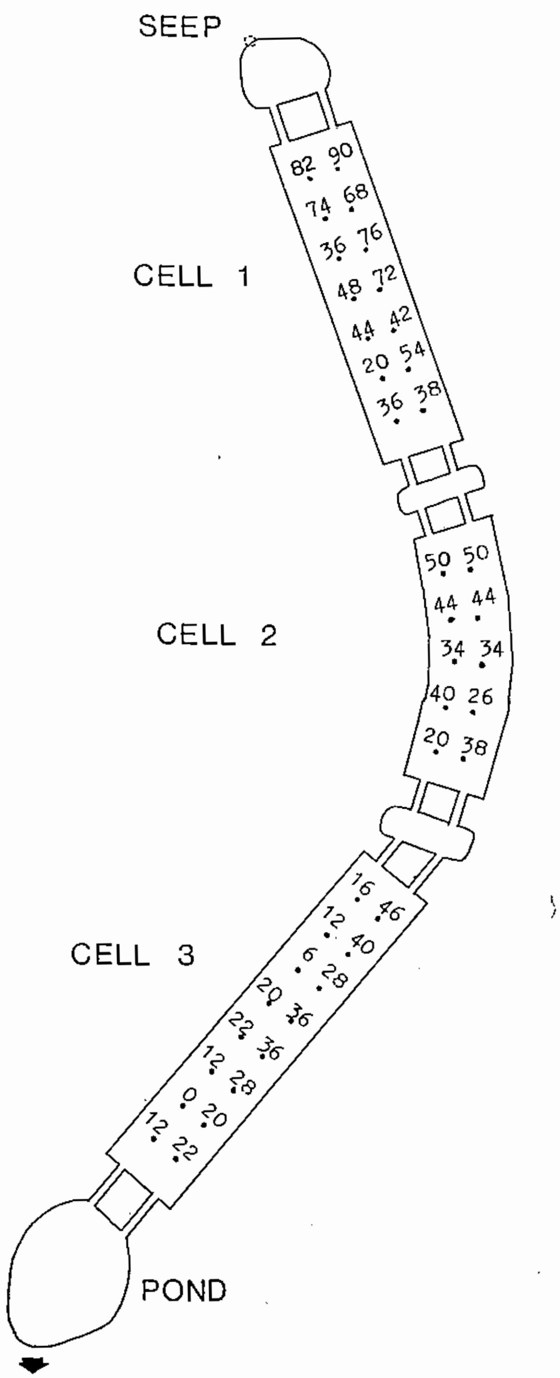

Figure 4.--Dissolved iron (mg/L) in water samples taken at 40 evenly spaced stations within the wetland, August 1987.

These three elements (i.e., the two higher plants and the algae) accounted for 87% of the total plant cover in 1986, and for 92% in 1987.

Total cover in 1986 was 45%; in 1987 it increased to 83%, in large part due to increased growth of the three elements above. The cutgrass (Leersia) was a relatively minor element of the vegetation in 1986, but in 1987 attained one-half the cover of the cattails. Cell 3 has the highest coverage of flowering plants, while Cell 2 cover increased dramatically in 1987. Algae were quite abundant in Cell 2, in part due to the frequent open spaces with reduced higher plant cover. The cattail cover in Cell 1 did not increase between 1986 and 1987; muskrats were present in this cell, fed on cattail rhizomes, and were trapped out intermittently.

Cattail shoot counts in August 1987 yielded the following estimates of shoot density per wetland cell (N is the number of 0.5-m^2 quadrats sampled): Cell 1: $6.5/m^2$ (N=8); Cell 2: $12.0/m^2$ (N=6); Cell 3: $18.7/m^2$ (N=9). Clearly cattails are more numerous in Cell 3.

Table 5.--Average total acidity (mg/L) by season of each wetland cell and ditch outfall; parenthetical values are the percentage reduction in acidity from the seep; N=5 for pre-wetland means, N=4-7 for post-wetland means.

Location	Pre-Wetland (1983-1985)	Winter 1985-6	Spring 1986	Summer 1986
Seep	207	95	52	92
Cell 1	---	82	38	57
Cell 2	---	76	36	32
Cell 3	---	70	26	15
Ditch End	187 (10%)	65 (32%)	22 (58%)	1 (99%)

	Fall 1986	Winter 1986-7	Spring 1987	Summer 1987	Fall 1987*
Seep	88	54	57	46	61
Cell 1	64	38	25	24	3
Cell 2	59	27	22	15	17
Cell 3	41	20	14	3	-4
Ditch End	20 (77%)	20 (63%)	15 (74%)	6 (87%)	-3 (100%)

* after site modifications of August 31, 1987

Winter = Dec-Feb
Spring = Mar-May
Summer = Jun-Aug
Fall = Sep-Nov

Table 6.--Coverage of plant and algal species by cell in 1986 and 1987. A "--" denotes absent, and a "+" denotes present but with less than 1% mean cover.

Species	Cell 1		Cell 2		Cell 3		Overall	
	1986	1987	1986	1987	1986	1987	1986	1987
Typha latifolia (cattail)	29	28	20	48	39	51	30	42
Leersia oryzoides (cutgrass)	2	8	1	20	6	35	4	22
Lemna minor (duckweed)	--	--	6	2	7	5	4	2
Equisetum arvense (horsetail)	--	--	--	--	1	+	+	+
Phalaris arundinacea (reed canary grass)	1	6	--	--	--	--	+	2
Polygonum sagittatum (tear-thumb)	--	--	--	--	1	+	+	+
Verbena hastata (vervain)	--	--	--	--	+	--	+	--
Salix sp. (willow)	--	--	--	--	+	--	+	--
Alisma sp. (water plaintain)	--	+	--	2	--	--	--	1
Ailanthus altissima (tree of heaven)	--	+	--	--	--	--	--	+
Epilobium sp. (willow-herb)	--	--	--	2	--	1	--	1
Algae*	9	12	--	24	5	4	5	12
TOTAL	41	55	27	98	60	97	45	83

*-including: mixed populations of _Oscillatoria_ sp.; 2 _Euglena_ sp.; _Chlamydomonas polypyrenoideum_; and _Microspora pachyderma_.

In the winter, 22 of 40 quadrats had visible algal populations, with 12 of these in Cell 3. Overall algal cover in the spring was 41%, with Cell 1 = 54%, Cell 2 = 30%, and Cell 3 = 37%. The algae are a prominent vegetational element on the substrate and water surfaces year-round.

Plant Tissue Metal Levels

Cattail roots at the Simco #4 wetland had the highest iron concentrations, followed in sequence of decreasing concentrations by outer rhizomes, entire rhizomes, leaf bases, leaf blades, and inner rhizomes (table 7). Control plants had higher iron values in the outer rhizome samples than found in the experimental (i.e., Simco #4 wetland) plants. Control root values were greater than three of six experimental values. Although no apparent surface mining has occurred in the immediate watershed where controls were taken, it is possible that some past disturbance has resulted in elevated iron levels in the substrate.

All roots and outer rhizome samples were iron-red and had iron encrustations on the surface that were difficult to remove from the epidermis. Inner rhizome tissues and hand-sectioned root tissues were not discolored. These observations correlate with the higher iron values observed in this study. Taylor et al. (1984) demonstrated that iron coatings (plaques) form on the surface of

Table 7.--Average iron and manganese concentrations (mg/g dry weight) in plant structures of *Typha latifolia* from the Simco #4 wetland and a control site near DuBois, PA; numbers of plants sampled in parentheses.

Structure	Location (Cell No.)	Fe Jan '87	Fe Apr '87	Mn Jan '87	Mn Apr '87
Leaf Blade	1	--	0.86(2)	--	0.47(2)
	2	--	1.02(2)	--	0.41(2)
	3	--	1.27(3)	--	1.86(3)
	Control	0.65(3)		1.07(2)	
Leaf Base (sheath)	1	14.69(1)	0.78(2)	0.02(1)	0.23(2)
	2	15.50(1)	0.92(3)	0.38(1)	0.13(3)
	3	--	1.20(2)	--	0.52(2)
	Control	0.26(3)		0.92(3)	
Inner Rhizome	1	0.55(4)	0.84(4)	0.12(4)	0.13(4)
	2	0.29(3)	2.70(2)	0.05(3)	0.07(2)
	3	0.33(4)	1.52(5)	0.21(4)	0.18(5)
	Control	0.39(1)		0.24	
Outer Rhizome	1	8.34(3)	22.16(2)	0.31(3)	0.18(2)
	2	12.77(3)	7.95(3)	0.05(3)	0.09(3)
	3	11.52(2)	2.51(2)	0.12(2)	0.27(2)
	Control	14.51(2)		0.41	
Entire Rhizome	1	3.61(4)	7.71(4)	0.06(4)	0.13(4)
	2	6.03(4)	1.73(2)	0.03(4)	0.09(2)
	3	2.46(3)	6.24(2)	0.12(3)	0.15(2)
	Control	0.60(1)		0.48(1)	
Root	1	9.11(4)	27.82(3)	0.11(4)	0.26(3)
	2	21.95(3)	41.65(2)	0.08(3)	0.10(1)
	3	107.04(2)	12.63(1)	0.20(2)	0.61(1)
	Control	21.40(2)		0.65(2)	

cattail roots when ferrous iron is oxided and is deposited on the roots and in the adjacent rhizosphere. Macfie and Crowder (1987) found that iron plaque formation in cattails was positively correlated with extractable iron in the substrate and pH. Consequently, higher iron values would be expected in surface tissues than in inner tissues.

Roots and rhizomes have higher iron concentrations than foliar tissues (table 7). Other researchers have found the same pattern in cattails (Taylor and Crowder 1983; Adriano et al. 1984). In contrast, foliar samples of cattails had higher manganese concentrations than rhizome and root samples (table 7). Adriano et al. (1984) found no seasonal patterns for manganese accumulation. However, their study demonstrated rhizomes had much lower concentrations of manganese than shoots.

Microbial Studies

Respectably high numbers of aerobic heterotrophs (the average viable cell count for the entire wetland was 1.4×10^8 colony forming units/mL sediment) occurred in the wetland. Typical trends in the numbers of aerobic heterotrophs through the wetland from the seep to the outfall are shown in fig. 5. An obvious bloom in the total population of aerobic heterotrophs (averaging about two orders of magnitude in increased population) was seen in the spring samples. Bacterial numbers were lowest in the winter samples. The highest viable cell counts were found on agar-water and brain heart infusion agar. To put these results into context over a 1-year period, the numbers of bacteria in the wetland samples were about two orders of magnitude lower than those found in rich agricultural soils, about the same as those found in eutrophic lakes, and three orders of magnitude higher than those found in oligotrophic lakes. However, species diversity of the aerobic heterotrophs was variable and relatively low over the wetland, averaging only between 9 and 25 distinct colony types. Also, iron- and manganese-oxidizing bacteria were quite variable (from none detected to over 10^8/mL of sample) through the wetland (data not shown). This was surprising because the decline in soluble iron from the seep to the outfall was relatively smooth, while the manganese content increased slightly. Black water with the characteristic smell of H_2S and that of mercaptans was noted, especially in

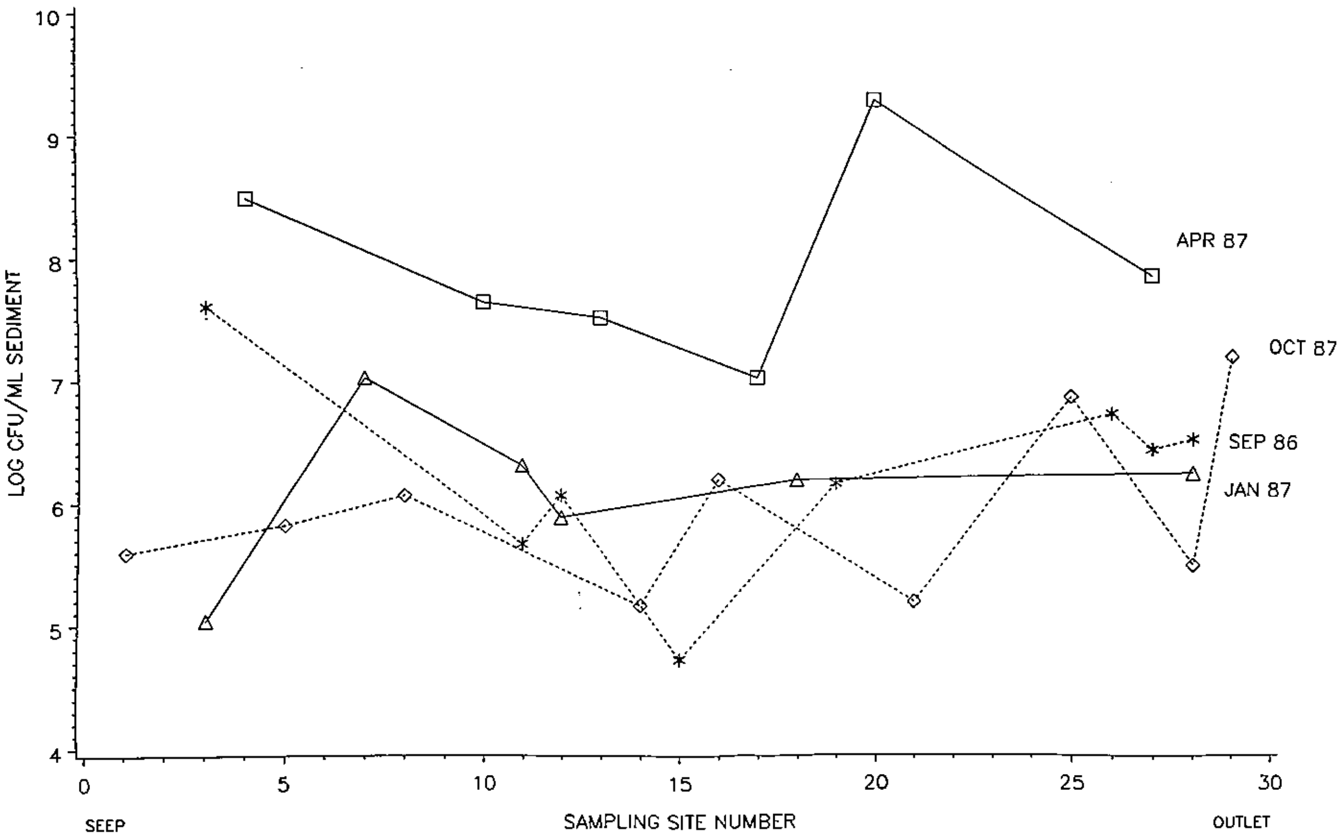

Figure 5.--Viable counts of microbes on agar water medium by location in the wetland expressed as the log of colony forming units per mL of sediment (CFU/mL). Sampling site 1 corresponds to the seep; 29 corresponds to the outlet.

association with bales of hay placed in the wetland to alleviate channelization.

DISCUSSION

Given the relatively high pH of this mine drainage, iron is expected to come out of solution to some degree and settle without the presence of a wetland. In addition, the elevated pH probably enhances the biological responses to metals at the cell surface (Campbell and Stokes 1985). Thus, the seep pH of 6.0, which is relatively unaltered by the wetland, positively influences the wetland with respect to iron. At this point, it is impossible to discriminate between the pH effect on iron solubility and the pH effect on biological surfaces and cation exchange. However, the effect of wetland installation on iron removal is significant: iron removal 2 years after wetland construction is up 80%-90% over pre-wetland iron removal. Why is this the case? There are a few conspicuous differences between pre- and post-wetland sites, and several wetland factors to consider regarding the wetland's apparent effectiveness:

(1) The area of water impoundment has been increased by wetland installation. As a result the amount of water exposed to the air is much greater.

(2) The retention time of the water was probably increased by wetland installation. Therefore, any biologically assisted or unassisted reactions leading to iron precipitation have a longer period in which to occur.

(3) Biological activity in the form of microbial, algal, and cattail-cutgrass populations is much greater following wetland construction and vegetation cover increased considerably from the first to the second summer. This activity was promoted principally by the addition of organically rich compost growth medium for the cattails. Cattails are highly productive and thereby return a considerable amount of organic material to the system via winter die-off of stems and leaves. Any effect of the substrate apart from biological activity cannot account for all of the iron removal. During the first winter (1985-6) following wetland construction little else but spent mushroom compost was in the wetland; the winter efficiency was 28%, approximating pre-wetland efficiency. However, during the second winter (1986-7) efficiency increased to 51%. This difference is probably attributable to biological activity.

(4) Cattail roots and rhizomes are directly absorbing or precipitating some of the iron. Roots from cattails at Simco #4 contained up to

5 times as much iron as cattail roots from a control site. Iron precipitates are also loosely associated with cattail roots and rhizomes. The cattail rhizosphere may be a conducive environment for aerobic microbes such as species of Thiobacillus that oxidize iron. Further study will determine whether the distribution of iron in the substrate of a wetland receiving mine drainage is related to proximity to roots.

(5) Wetland Cell 3 was the most effective portion of the constructed wetland in improving the water quality. It is the largest cell, but the degree of water improvement is greater than can be accounted by size difference. Other characteristics of Cell 3 include the highest total flowering plant cover, the greatest density of cattail stems, and cutgrass coverage exceeding the other two cells. Microbial populations were no greater in Cell 3 than in the other cells. Cell 3 receives water with the lowest level of iron, and thus its higher efficiency may, alternatively, reflect (a) a water pretreatment effect of previous cells, or (b) that iron is more easily removed by a wetland at lower incoming levels.

(6) The diversion ditches and hay bale dikes installed in the fall of 1987 had an immediate effect on iron removal (increased), pH (decreased), manganese (increased), and acidity (decreased). It is likely that hay, much like sawdust, encourages anaerobic sulphate-reducing bacteria that precipitate FeS - FeS_2 (Tuttle et al. 1969). On the downstream side of hay bales conspicuous areas of black water were observed, consistent with this hypothesis.

(7) The roles of algae in this wetland are not yet understood, but their ubiquitous presence, both in the floating iron surfactant/flocculant (primarily Oscillatoria) and at the substrate-water interface, deserves further study.

(8) Little is known about heterotrophic bacteria in acidic mine drainage. However, they have been shown to be involved in ferric iron reduction and in iron deposition in freshwater lakes (Jones 1986). Aerobic and facultatively anaerobic bacteria are thought to contribute to iron and possibly manganese removal primarily through reduction of these metallic species. Ferric iron could be reduced to iron oxide (FeOOH), which is believed to be the reactant with HS^- that leads through mackinawite to greigite and finally to pyrite (see Jones 1986 and references therein). The HS^- is produced anaerobically by several species of bacteria. Anaerobic bacteria have not yet been counted in the wetland, but as noted previously, the products of sulfate reduction by bacteria were obvious at each of the visits to the wetland. The oxidation of iron was evident in the wetland, as indicated by the deposition of $Fe(OH)_3$ and the formation of yellow-boy. This was especially evident around the shallow outlets and inlets of the seep and each cell. These areas were undoubtedly the most aerobic zones in the wetland. Iron-oxidizing bacteria of the genus Thiobacillus were found throughout the wetland.

(9) The winter efficiency of wetlands receiving mine drainage has frequently been questioned. Data from Simco #4 indicate only a slight reduction in iron-removal efficiency between summer and winter (56% to 51%). This result is encouraging, but the water quality and other site-specific conditions prohibit generalizations at this point.

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