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The use of sulfur isotopes to monitor the effectiveness of constructed wetlands in controlling acid mine drainage

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Abstract Acid drainage containing high levels of iron, manganese, and sulfate is a pollution problem widely associated with abandoned coal mines. The remediation of these sites commonly falls to public agencies, so policy-makers need to allocate scarce resources over many sites and to find cost-effective remediation strategies. In recent years, constructed wetlands have become a popular alternative to more labor- and capital-intensive treatment systems. Within these wetlands, bacterial sulfate reduction occurs and is often cited as an important remediation process, but few measurements of reduction rates have been made. We studied the Wills Creek site in NE Ohio, a series of staged constructed wetlands, to evaluate the use of stable isotopes of sulfur as a rapid technique to assess the effectiveness of wetland design. Sulfur isotopes are widely used in marine sediments to measure the rates of bacterial processes, but have not previously been applied to constructed wetlands. Water chemistry measurements indicated that the wetland system as a whole was effectively removing acidity and iron, but not sulfate. We found that sulfate reduction rates were slow relative to the sulfate loadings and that therefore sulfate reduction was ineffective as a remediation process for sulfate contamination in constructed wetlands designed like those at Wills Creek, in agreement with the five-year record of water chemistry measurements. Moreover, our results showed that cells especially constructed to enhance sulfate reduction in fact showed slower reduction rates than more typical compost wetland cells. We conclude that the concept of staged wetlands, although intuitively appealing, is no more effective than a series of ordinary wetlands of the same size. This study illustrates that stable isotopes of sulfur can be a quick and cost-effective way of assessing the functioning of bacterial remediation processes in constructed wetlands and could be used by regulatory agencies to evaluate various design strategies.

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1
The problem of acid mine drainage

Acid mine drainage is formed when sulfides (commonly pyrite) from coals and from shales associated with coal beds oxidize by coming in contact with oxygenated water, releasing acid, sulfate, and metals such as Fe, Mn, and Al into the water. The acidic conditions and elevated dissolved metals associated with mine drainage can be toxic to aquatic life and, if introduced into residential wells, potentially harmful to humans. Surface and groundwaters in areas with abandoned coal mines commonly exceed Secondary Maximum Contaminant Limits set by the USEPA for acidity, Fe, Mn, and sulfate. Not all coal-bearing sequences produce significant acid drainage, however. The amount and depositional nature of the pyrite present are important factors. If the coal was formed in a freshwater environment, as is common in the western United States, the sulfur content is generally low and significant acid mine drainage is not produced. However, coal beds formed within marine sequences, such as in the Ohio and West Virginia portion of the Appalachian region, contain several percent of pyrite sulfur and can produce significant acid mine drainage (Langmuir 1997). Also important is the size of pyrite crystals: the smaller the size, and thus the greater the surface area, the faster the pyrite oxidizes. Pyrites formed in marine environments are generally smaller than pyrites formed in freshwater environments (Caruccio and Ferm 1974) and so react faster.

Other factors influencing the amount of acid mine drainage include the relationship of the coal bed to carbonate rocks, which can neutralize acid mine drainage, and the relationship of the coal bed to the water table: Coal beds above the water table can be oxidized faster than those below the water table.

The processes of pyrite oxidation have been reviewed extensively by Stumm and Morgan (1981). The dominant reactions that take place are

$$FeS_2 + 7/2O_2 + H_2O = Fe^{2+} + 2SO_4^{2-} + 2H^+ \quad (1)$$

$$Fe^{2+} + H^+ + 1/4O_2 = Fe^{3+} + 1/2H_2O \quad (2)$$

$$Fe^{3+} + 3H_2O = Fe(OH)_3 + 3H^+ \quad (3)$$

These reactions show that both the iron and the sulfide in pyrite oxidize and release acidity. The breakdown of pyrite is one of the most acidic of all weathering reactions because the oxidation of one mole of pyrite releases four moles of H^+ (Webb and Sasowsky 1994).

In addition to reacting with oxygen and water, pyrite can be oxidized by dissolved ferric iron according to the following reaction (Stumm and Morgan 1981):

$$FeS_2 + 14Fe^{3+} + 8H_2O = 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \qquad (4)$$

In equation 4, free oxygen is not needed so that pyrite can continue to be oxidized by ferric iron alone. Even if the supply of atmospheric oxygen is completely cut off, the dissolution of any solid $Fe(OH)_3$ can supply the necessary ferric iron (Stumm and Morgan 1981; Moses et al. 1987). Thus if the oxygen supply is eliminated by flooding or sealing mines, the production of acid mine drainage can still continue once reactions have begun (Webb and Sasowsky 1994). Accordingly acid production from old mines is a persistent problem. Moreover, many of these mines have been abandoned and it has fallen to the public sector to provide remediation of the acid drainage problems they cause. Traditionally, acid mine drainage was treated chemically, but chemical processes are expensive and chemical byproducts may themselves be damaging to aquatic life. Limestone treatment, which raises alkalinity, is popular because of its lower cost, good buffering ability, and lower potential for overdosing. However, if conditions are aerobic, iron and aluminum can precipitate and coat the limestone, hindering its dissolution and rendering it useless (Brodie et al. 1993).

Cost-effective remedies for long-term control of acid drainage are called for. In the 1970s, it was observed that some natural wetlands, rather than being degraded by acid drainage, actually exhibited some cleanup of the water. Using this observation, the Tennessee Valley Authority (TVA) began experimenting with artificial *Typha* (cattail) marshes to treat acid mine drainage. Most designs of the wetlands subsequently constructed to treat acid mine drainage have used *Typha* surface-flow marshes (Girts 1986; Bastian and Hammer 1993).

Interest in using wetlands for the remediation of polluted waters (acid mine drainage, sewage, industrial and agricultural runoff) has been increasing because of their natural and economical way of providing a sink for heavy metals, raising the pH, and filtering suspending materials (Brix 1993; Witthar 1993; Berezowsky 1995). Wetlands are ineffective, however, if the input pH is too low. They behave best if the influent water is net alkaline. Therefore the preferred design is to precede the wetland by a covered trench lined with crushed limestone, termed an anoxic limestone drain or ALD (Kleinmann et al. 1998). Keeping the limestone isolated from the atmosphere prevents coating by iron-oxide scale and allows the limestone to continually add alkalinity. Thus a wetland or series of wetlands preceded by an anoxic limestone drain can provide an ideal, low-maintenance remediation strategy for acid mine drainage. Within this framework, however, many design alternatives are possible. A way to quickly assess long-term performance of different designs is needed.

2
Sulfate reduction as a remedial process in wetlands

One favorable geochemical property of wetlands is that they foster bacterial sulfate reduction. Laboratory and pilot scale tests have proven that sulfate reduction by bacteria in wetlands is effective in raising pH and removing metals and sulfate from acid mine drainage (Hedin et al. 1989; Hammack and Edenborn 1992; Dvorak et al. 1992; Ledin and Pedersen 1996). There is one field study that we are aware of that has measured sulfate reduction rates: McIntire and Edenborn (1990) used radioactively-labeled sulfur as a tracer and measured rates high enough to have a significant effect on effluent sulfate concentration, but found that sulfate reduction rates were highly variable.

Sulfate-reducing bacteria are a group of prokaryotic microorganisms that use electron donors to reduce sulfate by dissimilatory sulfate reduction. In dissimilatory sulfate reduction the electrons are used for energy production whereas in assimilatory reduction the electrons are used to build biological compounds (Singleton 1993). Sulfate-reducing bacteria use the electrons to reduce SO_4^{2-} while oxidizing an organic substrate (Singleton 1993). Dissimilatory sulfate reduction is the major terminal degradation process in anaerobic environments. Evidence for the presence of sulfate-reducers is blackened sediment, which results from the precipitation of iron sulfides, and by the smell of hydrogen sulfide (Fauque 1995).

The oxidation of organic compounds, using CH_2O as a model, coupled with sulfate-reduction and the precipitation of sulfides is shown in the following equations, where Me^{2+} are metal cations (Bjorn et al. 1996):

$$2CH_2O + SO_4^{2-} + H^+ = 2CO_2 + 2H_2O + HS^- \qquad (8)$$

$$Me^{2+} + HS^- = MeS + H^+ \qquad (9)$$

According to (Dvork et al. 1992), the following conditions should exist for significant removal of sulfate to occur by sulfate reduction:

(1) absence of dissolved oxygen,
(2) a source of sulfate,
(3) a source of organic carbon,
(4) the presence of sulfate-reducing bacteria,
(5) a way to physically retain metal sulfide precipitates, and
(6) a pH above 5.

If it meets these requirements, a wetland should be able to promote sulfate reduction. Acid mine drainage provides a source of sulfate; *Typha* wetlands can provide anaerobic conditions, a source of organic carbon, and the sulfate-reducing bacteria. The cattail stalks also provide baffling of water flow and physical retention of metal precipitates. If an anoxic limestone drain is used ahead of the wetland, a initial pH of ~5–6 can be maintained.

Meeting this set of conditions ensures that sulfate reduction will take place, but for remediation to be effective, the rate of sulfate reduction must be significant. The rate of reduction by bacteria depends on temperature, sulfate concentration, and electron donor. Studies by Kaplan and Rittenberg (1964) have shown that temperature and electron donor (ethanol or lactate in their study) influence the rate of sulfate reduction more than does sulfate concentration. Temperature affects the rate of reduction by encouraging or inhibiting bacterial metabolism. Under warmer conditions, where bacterial growth is more rapid, there is more sulfate reduction. With colder temperatures, bacterial growth is inhibited, and the sulfate reduction rate decreases. Therefore, a seasonal variation of sulfate reduction should be observed, given a relatively constant carbon source (the electron donor) and dissolved sulfate level.

3
The use of sulfur isotopes to determine the efficiency of sulfate reduction

The stable isotopes of sulfur have not been used in wetland studies, although they are commonly employed as tracers in the marine environment (Bruchert and Pratt 1996). Because wetlands receiving acid mine drainage share with the marine environment a high level of dissolved sulfate, sulfur isotopic studies have great potential for helping us understand wetland processes.

Sulfur is found in two dominant stable isotopic forms, ^{34}S and ^{32}S. Bacterial reactions favor the lighter isotope (^{32}S) so that a fractionation is produced in going from a sulfate reactant to sulfide products. According to Harrison and Thode (1958), bacteria such as *Desulfovibrio desulfuricans* fractionate sulfur in two main stages: (1) the entrance of sulfate into the cell, resulting in a small isotopic shift and (2) the breaking the S-O bonds, yielding a larger isotopic shift. Harrison and Thode (1957) also demonstrated that ^{32}S-O bonds are broken more readily than ^{34}S-O bonds. Therefore, bacteria prefer the lighter sulfur isotopes, producing H_2S that is depleted in ^{34}S relative to original sulfate. The degree of isotope fractionation by bacteria is inversely proportional to the rate of sulfate reduction (Kaplan and Rittenberg 1964; Goldhaber and Kaplan 1975). That is, slow-growing bacteria will have a slow rate of reduction and a large degree of isotope fractionation between the sulfate and sulfide, whereas fast-growing bacteria will have a fast rate of reduction with a small degree of isotope fractionation (Fig. 1).

Consequently, it should be possible to use the degree of fractionation between influent sulfate sulfur and the sulfide sulfur in the wetland soils to assess the rate of sulfate reduction in the wetland. Such an approach would be superior to measuring influent and effluent sulfate concentrations because the isotopes integrate reaction rates over time whereas the water chemistry provides only an instantaneous snapshot. The isotopic technique would also allow pinpointing the places in the wetland where reduction is more or less efficient.

4
The Wills Creek wetland as a case study of effectiveness of sulfate reduction

Sulfate reduction is a potentially important process in remediation of acid mine drainage by constructed wetlands, but our knowledge of how effective such wetlands are in promoting sulfate reduction, and hence our ability to improve their design, is limited. For the most part, we only have data on dissolved sulfate concentrations in the influent and effluent waters, but no detailed information on processes within the wetlands. We studied a constructed wetland in the Muskingum River watershed, the Wills Creek wetland, to elucidate details of the bacterial sulfate reduction process, using for the first time stable isotopes of sulfur as a tracer of bacterial activity.

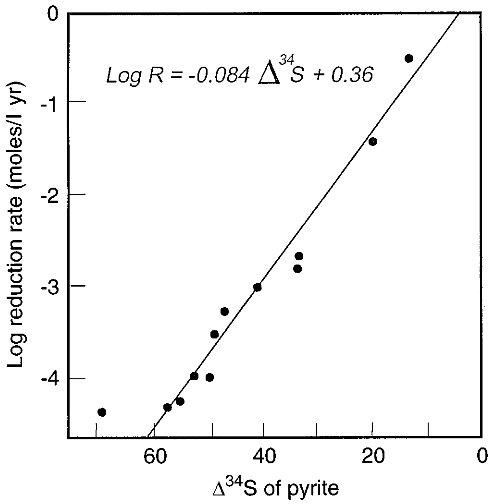

Fig 1. The relationship of sulfur isotope fractionation (between $\delta^{34}S$ sulfate and $\delta^{34}S$ sulfide) and rate of sulfate reduction by bacteria in marine systems where $\delta^{34}S$ sulfate = +20 permil (Maynard 1980

4.1
The history of the Wills Creek wetland

The study site (Fig. 2), the Wills Creek wetland, lies in Linton township along the southeast border of Coshocton and Muskingum Counties in the central part of Ohio. The wetland drains into Wills Creek Lake, which in turn feeds into the Muskingum River, the master stream in this county. The wetland was built in 1994 by the Division of Reclamation of the Ohio Department of Natural Resources, to remediate acid mine drainage from underground abandoned coal mines in the area. The deep mines were abandoned in the 1960s, after which strip mining became predominant.

The Special Studies Section of the Abandoned Mined Lands program (Division of Reclamation, Ohio Department of Natural Resources), began a six month investigation of the area in 1987 to determine the sources of acid mine drainage and to identify those areas needing immediate remediation. The study determined that the largest sources of acid mine drainage came from underground mines, and that reclaimed strip mines showed better water quality than underground mines (Brockmeyer 1987). Therefore, the focus of the Division of Reclamation was to remediate underground mines. The Wills Creek wetland was built to lessen the environmental impacts of a priority 2 site, as assessed by the National Abandoned Mined Lands program. (A priority 2 site is defined as one where the effects of past mining practices directly affect the health, safety, and or general welfare of the public.) The acid mine drainage around the site had infiltrated into the ground-water, contaminating the wells of several

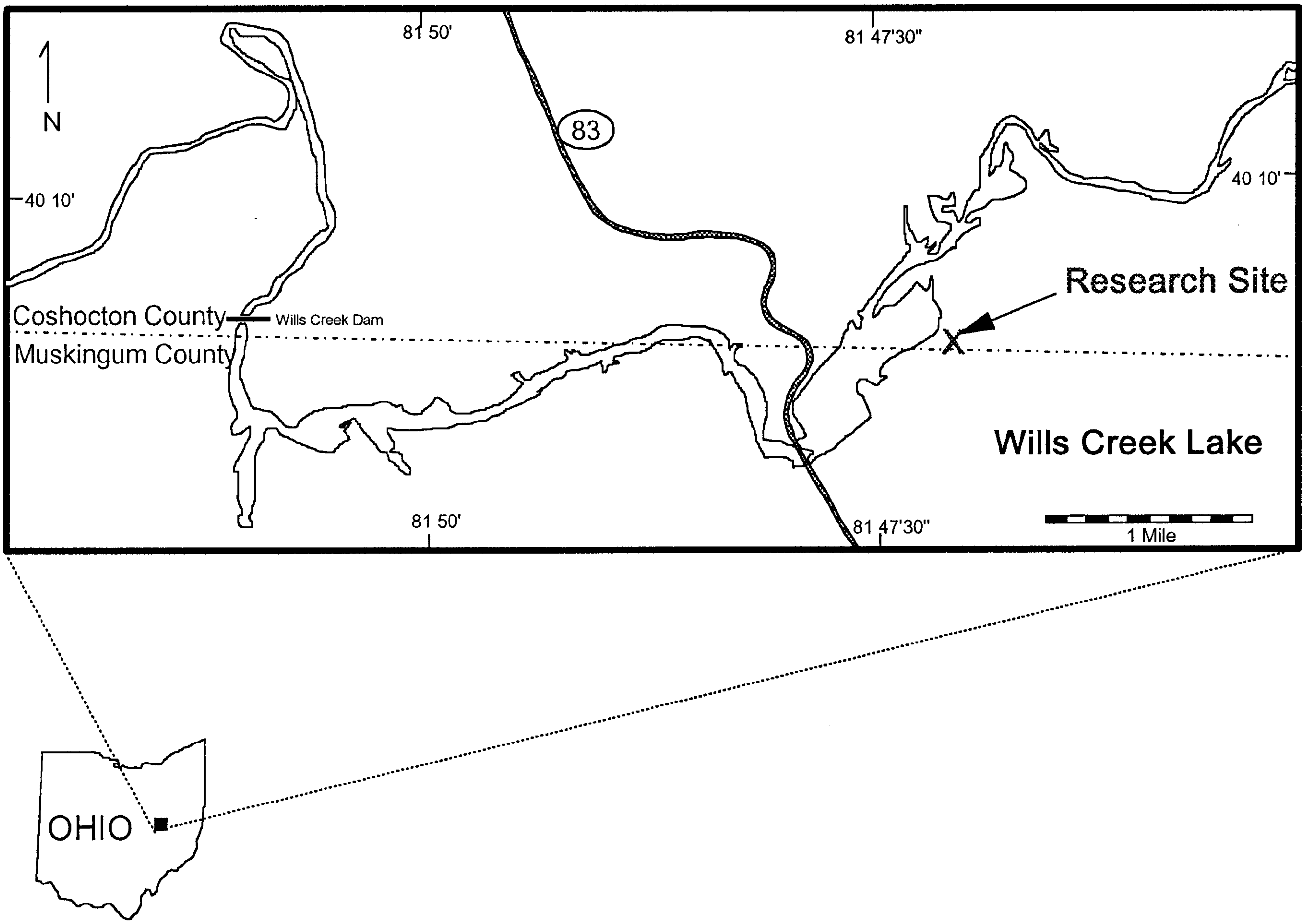

Fig. 2. Location of the Wills Creek wetland

occupied residences. The main purpose of the wetland was to remediate the ground-water (Brockmeyer 1987).

The geologic host for the coals producing acid mine drainage in the Wills Creek area is the Allegheny series of Pennsylvanian age. The Middle Kittanning coal bed from this series was the target of active underground mining prior to the 1960s. The coals are in a marine sequence, thus the mines characteristically contain large amounts of iron sulfide sulfur that create significant acid mine drainage (Table 1). The thickness of this coal seam ranges from two to four feet. In Coshocton County, the total area underlain by the Middle Kittanning coal and clay is 86.8 square miles. The total area underlain by this coal seam in Linton Township is 15.6 square miles (Geology of Coshocton 1954).

Table 1. Representative analysis of the Middle Kittanning coal

	As Received	Moisture Free
Carbon	71.34	74.60
Hydrogen	5.56	5.30
Oxygen	12.85	9.38
Nitrogen	1.28	1.34
Sulfur	3.61	3.77
Ash	5.36	5.61
Total	100	100

Source: Geology of Coshocton County, 1954

The Wills Creek wetland is staged and consists of an aeration area, a settling pond, and a series of three cells (Fig. 3). The settling pond and cells 1–2 are surface flow systems. Cell 3 has a subsurface flow system, used to promote anaerobic conditions. Cells 1–2 are shallow and planted with *Typha*, whereas the settling pond and cell 3 are deeper and unvegetated (Table 2). Anoxic limestone drains are used to pretreat the acid mine drainage before it is exposed to the atmosphere in the aeration area and settling pond. Between each cell, an aerobic shallow channel, approximately one foot in width by two to three inches deep, feeds acid mine drainage into the following cell, except for cell three, which is fed by an underground, anaerobic pipe. The settling pond, each cell, and the channels connecting the cells are lined with landfill liner. According to the Department of Natural Resources, the wetland was designed to have a retention time of three to five days.

Ohio Department of Natural Resources measurements of water quality for the period 8/94 through 10/95 permit calculation of wetland efficiency over the first two years of operation (Table 3). For iron, influent concentration averaged 23.5 mg/l while the effluent concentration was 0.8 mg/l, giving a removal efficiency of 97%. For sulfate, the removal rate is much less. Concentrations were 560 mg/l (in) and 515 mg/l (out) for a removal efficiency of 8%. Our measurements of sulfate over the period 7/95

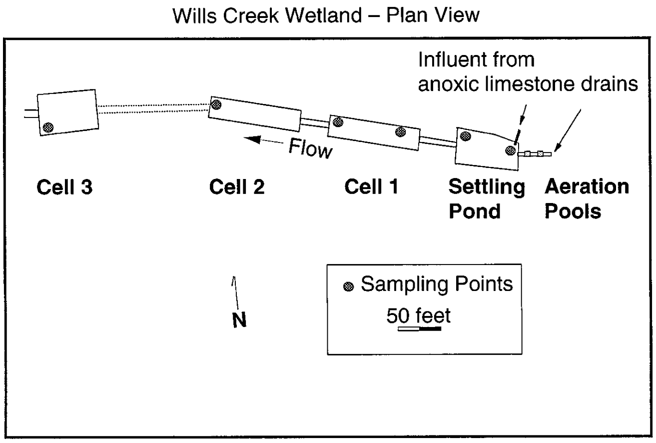

Fig. 3. The layout of the Wills Creek wetland into three different cells. The original design was to have all of the input flow through the aeration pools, but additional seeps were discovered after construction began and these were fed directly into the settling pond through anoxic limestone drains

Table 2. Dimensions of the Wills Creek constructed wetland cells

Cell	Surface area (m^2)	Water depth (m)	Water volume (m^3)	Initial substrate
Settling pond	314	1.52	478	
Cell 1	255	0.08	19.5	mushroom compost/lime
Cell 2	255	0.08	19.5	manure/lime
Cell 3	312	0.76	238	limestone/ Fermway[a]

Total volume = 755m^3; surface area of composted cells = 822 m^2

[a] Fermented mixture of chicken/cow manure

Table 3. Water chemistry in Wills Creek wetland system. 10/94 through 10/95 data from OH Div of Reclamation (Hsu 1998) Later data by authors. A blank indicates not measured

	pH	Conductivity	Fe ppm	Mn ppm	SO_4 ppm	SO_4/S.C.
10/25/94						
ALD	6.15	1422	24	1.21	570	0.401
Cell 1 outlet	6.9	1351	6.71	1.55	550	0.407
Cell 3 outlet	7.09	1349	0.08	0.4	482	0.357
12/19/94						
ALD	6.18	1780	40	1.68	640	0.360
Cell 1 outlet	6.82	1450	11.1	1.47	615	0.424
Cell 3 outlet	6.92	1420	1.44	0.78	585	0.412
5/22/95						
ALD	6.72	1740	28.1	3.895	645	0.371
Cell 3 outlet	7.13	1350	1.21	1.74	546	0.404
8/1/95						
ALD	6.65	1149	8.32	1.01	396	0.344
9/1/95						
Cell 3 outlet	7.10	1240	0.95	0.79	488	0.394
10/4/95						
ALD	6.35	1204	17.1	1.98	547	0.454
Cell 3 outlet	7.00	1179	0.41	0.44	474	0.402
3/14/96						
ALD	6.62				581	
Cell 11 outlet	6.47				624	
Cell 3 outlet	6.61				815	
8/21/96						
ALD					313	
Cell 1 outlet					296	
Cell 3 outlet					315	
3/20/97						
ALD	5.95				224	
Cell 1 outlet	5.80				254	
Cell 3 outlet	6.40				241	
4/30/98						
ALD	6.07				516	
Cell 1 outlet	6.07				616	
Cell 3 outlet	5.94				607	
6/7/99						
ALD	5.85	1293			494	0.382
Cell 1 inlet	3.18	1815			1086	0.598
Cell 1 pore water	5.85	1795				
Cell 2 inlet	3.28	1689			914	0.541
Cell 3 outlet	3.28	1596			844	0.529

through 6/99 show that sulfate efficiency subsequently fell even lower. Thus the long-term record of water chemistry shows that the Wills Creek wetland system has been ineffective at remediation of sulfate. In our study we compare the results from sulfur isotopic measurements to the water chemistry record of wetland performance.

4.2
Study methods

We took soil and water samples over a five-year period, on 7/25/95, 3/14/96, 8/21/96, 3/20/97, 4/30/98, and 6/07/99. Sampling intervals were designed to provide data from dormant season (early spring) and productive season (mid-summer) periods. Soil grab samples were taken at the surface and approximately six inches below the surface. During the 6/99 round, samples were taken from four vertical profiles using a post-hole digger to test vertical variability. Approximately 250 ml of surface water was taken from each cell during every sampling round. Discharge of each cell was taken in April 1998 during a high rain month to determine minimum retention time.

Water samples were analyzed for dissolved sulfate concentration and for sulfur isotopic composition of sulfate. The soil samples were used to determine sulfur isotopes of sulfides, total and organic carbon, total sulfur, and sediment mineralogy.

Dissolved sulfate was determined by precipitation with $BaCl_2$ (Standard Methods 1971). The resulting $BaSO_4$ precipitate was then analyzed as SO_2 on a Nuclide 6–60 mass spectrometer at Indiana University for sulfur isotope determination. Sulfide was extracted from the soil samples using the method of Canfield et al. (1986) and analyzed for sulfur isotopes using the methodology of Bruchert and Pratt (1996). In this procedure, sulfide sulfur is extracted in two forms: acid volatile sulfur and chrome-reducible sulfur. Acid volatile sulfur (AVS), which is dominantly from FeS, is released as H_2S by the addition of 6N HCl. Subsequently, chrome-reducible sulfur (CrRS), mostly from FeS_2, is converted to H_2S by reaction with $CrCl_3$. In each case, the H_2S is precipitated as Ag_2S, which was then converted to SO_2 gas for isotopic measurement.

The sulfur isotopic results are reported using the delta notation where

$$\delta^{34}S = [(^{34}S/^{32}S)\text{sample} - (^{34}S/^{32}S)\text{standard}]/(^{34}S/^{32}S)\text{standard} \times 1000.$$

The reference standard is Canyon Diablo Troilite (CDT), which is an iron meteorite that represents a bulk earth sulfur isotopic composition (Faure 1986) and the units of measurement are permil.

To determine if crystalline minerals, especially iron sulfides and iron oxides, were forming and remaining immobilized in the soil, we determined the mineralogy of the soil by X-ray diffraction . We used the random powder method of Moore and Reynolds (1989) to analyze the soil samples for iron oxides, sulfides, sulfates, and clay minerals on a Siemans D500 diffractometer with copper radiation.

Water samples from the Wills Creek wetland were also taken by the Division of Reclamation, Ohio Department of Natural Resources for all the sections of the wetland except cell 2. Samples were analyzed by Coshocton Environmental Testing, Inc. To aid in the interpretation of the water chemistry, the data was run on PHREEQC, a geochemical equilibrium program developed by the U. S. Geological Survey (Parkhurst 1995). Discharge was calculated from measurements of flow rate, using a Model 2100 Current Velocity Meter by Swoffer Instruments, Inc., and the cross sectional area of the inter-cell channels.

4.3
Results

Our results show that the Wills Creek design was not effective in removing sulfate from the acid mine drainage and that sulfate reduction rates were slow. Aqueous sulfate concentration levels in the cells of the wetland ranged from about 200 to 1000 mg/l (Table 3), with the variation proportional to the salinity of the water as measured by specific conductance. There was little variation from cell to cell for a given sampling round except the final sampling in June of 1997, which showed about a 10% lowering of sulfate in each cell. Sulfur concentrations in the soil show that sulfide levels are relatively low. Every cell has about 1% total sulfur, with the average being 1.28 ± 0.31 (95% confidence interval). About 2/3 of this S is in the form of sulfide: 0.84 ± 0.34%. Total carbon was 9.52 ± 2.11%, which was made up dominantly of organic carbon at 6.66 ± 1.65%. The difference, or 2.86%, would be acid-reactive carbonate carbon, which would add some buffer capacity to the system.

During the first two years of wetland operation, 1994–1995, sulfate levels of the water declined slightly in passing through the system. Average inlet concentration was 560 ppm vs 515 ppm at the outlet from cell 3 (Table 3). From 1996 on, however, inlet sulfate was actually less than outlet sulfate (Table 3). For the whole period of record, 1994–1999, inlet sulfate averaged 493 ± 140 ppm, while outlet sulfate averaged 540 ± 190 ppm. The confidence intervals of the means overlap, so the wetland system may not actually have been exporting sulfate after 1996, but rates of sulfate removal are too low to measure by mass balance.

Sulfur isotopes showed a large fractionation indicating slow reduction rates. The sulfur isotope fractionation between sulfate and sulfide is the difference in $\delta^{34}S$ value for dissolved sulfate and $\delta^{34}S$ value for iron sulfides, either FeS (AVS) or FeS_2 (CrRS). The $\delta^{34}S$ for SO_4 ranged from −2.41 to −5.12 with an average of −3.36 permil. The values were virtually the same in all the cells for all sampling events (Table 4). Sulfide values in surface soil ranged from −7.69 to −44.06 permil. The average for AVS was −28.2 and for CrRS was −30.6. The two sulfide means were found to be statistically identical (Hsu, 1998). Because CrRS (pyrite) is the stable end product, we used the CrRS values to determine the fractionation between sulfate and sulfide. Then the amount of fractionation between $\delta^{34}S$ sulfate and $\delta^{34}S$ FeS_2 can be used to determine the rate of bacterial sulfate reduction. Weighting each cell equally, the average fractionation or spread for the Wills Creek wetland system was 27.2 ± 9.4 permil (Table 4). There are slight differences between the early spring (dormant season), which averaged a spread of

Table 4. Sulfur isotope values (per mil, CDT) of sulfides, Wills Creek (top 20 cm of soil)

	Sulfate	AVS	CrRS	Fractionation (Sulfate – CrRS)
7/24/95				
Settling Pond	−2.57			
Cell 1, A2	−2.44	−13.20	−12.32	9.88
Cell 2	−3.14			
Cell 3	−2.46	−28.99	−30.11	27.65
3/13/96				
Settling Pond	−4.47			
Cell 1, A2	−3.22	−21.26	−20.73	17.51
Cell 2	−3.52			
Cell 3	−4.34	−36.47	−39.45	35.11
8/20/96				
Settling Pond	−3.56			
Cell 1, (n = 2)	−3.50	−14.28	−16.37	12.87
Cell 2	−5.12		−44.06	38.94
Cell 3	−3.85	−30.87	−33.78	29.93
3/19/97				
Settling Pond	−3.56			
Cell 1, (n = 2)	−3.35	−29.29	−25.88	22.53
Cell 2	−3.09	−41.04	−43.12	40.03
Cell 3	−2.41	−32.05	−30.82	28.41
6/7/99				
Settling Pond				
Cell 1 (n = 4)	−3.62	−26.51	−29.66	26.04
Cell 2	−3.47	−21.86	−23.77	20.30
Cell 3	−2.72	−33.13	−34.48	31.76
Cell 1 avg	−3.23	−20.91	−20.99	17.76
Cell 2 avg	−3.69	−31.45	−36.98	33.29
Cell 3 avg	−3.16	−32.30	−33.73	30.57
System avg	−3.36	−28.22	−30.57	27.21

20.0 ± 3.5 permil, and the late summer (active season), which averaged 16.3 ± 7.9 permil. This seasonal difference was much more pronounced in the time period 1995–1997, when the summer spread was only 11.4 permil. There is a time trend to increasing fractionation in cell 1 (Table 4). For 1995–96, averaging summer and winter, fractionation was 13.7 permil; for 1996–97 the value rose to 17.7 permil; for the 1999 summer season, the value had risen still higher to 26.0 permil. These increases in fractionation correspond to the fall-off in wetland performance as measured by the change in the ratio of sulfate in/out to values greater than 1 after 1996.

Maynard (1980) calculated for sulfate in marine sediments that the reduction rate, R, in moles/(liter of pore water × yr), is given by

$$\mathrm{Log}R = -0.084\mathit{\Delta}^{34}\mathrm{S} + 0.36,$$

where $\mathit{\Delta}^{34}$S is the isotopic fractionation:

$$\mathit{\Delta}^{34}\mathrm{S} = \delta^{34}\mathrm{S}_{\mathrm{sulfate}} - \delta^{34}\mathrm{S}_{\mathrm{sulfide}}$$

Applying this result to the average spread of 27.2 permil, we find that the surface soil (the portion in communication with the overlying water) in the Wills Creek wetland system is reducing sulfate at a rate of 0.012 moles/(liter × yr). If the soil porosity is typical of wet sediment, about 80%, then this rate would be 0.00009 moles/(cm^2 × yr).

Fractionation also varied strongly among the cells. Values were available from cells 1 and 3 for all five sampling events; for cell 2 only for the last three events. Figure 4 shows the mean and 95% confidence interval for the three cells. The average fractionation for cell 1 was 17.8 permil, whereas cell 3, the cell designed to facilitate sulfate reduction, showed a much larger fractionation of 30.6 permil with no overlap in the ranges for the two cells. The three determinations for cell 2 averaged 33.3 permil, and its range overlapped both cells 1 and 3 (Fig. 4). Expressed as reduction rates, cell 1 was reducing sulfate at nearly 12 times the rate of cell 3. A change in the relative behaviors of cells 1 and 2 was seen in 1999 with the values converging. δ^{34}S values for cell 1 became lighter, whereas for cell 2 they became markedly heavier, indicating that the rate of sulfate reduction was slowing in cell 1, but increasing in cell 2. The strong seasonal contrast seen earlier in cell 1 also seems to have diminished or disappeared. Cell 3 maintained its very light values in the 1999 sampling round, indicating that its sulfate reduction rate remained very low.

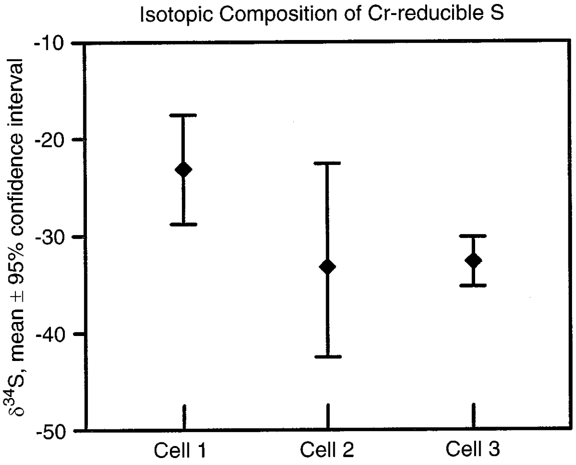

Fig. 4. Sulfur isotope values for the individual cells in the Wills Creek wetland, showing the range of variation. Much of the variation in cell 1 is associated with decreases in fractionation in the summer compared with winter for the years 1995–1997

Within-cell variations in fractionation were studied in cell 1. Vertically, the cell was found to be stratified, with a surface layer of oxidizing, bright orange sediment underlain by gray or black soil. The orange layer is 20 cm thick near the inlet at station A and thins steadily to 1 cm at station D (Fig. 5). The samples used in the averages above were all taken from just beneath this orange surface layer. δ^{34}S of AVS increases sharply with depth at each sampling station (Table 5); δ^{34}S of CrRS also tends to increase but not as markedly. Samples from the first 10 cm below the orange layer fall in the -40 to -20 permil range for AVS, whereas deeper samples give values of -10 to + 10 permil. CrRS slightly lighter than AVS for the surface samples, but still falls in the same -40 to -20 range. In deeper samples, however, CrRS is much lighter than AVS, falling in the -2 to -32 permil range. Horizontally, there is a steady decrease in the isotopic composition of the AVS of the top-most samples from about -20 in the first half of cell 1 to as low as -38 at the last position measured. CrRS follows the same trend except for station A2, which is anomalously light. The shift to heavier sulfur isotopic values with depth is consistent with the pattern seen in modern marine sediments (Goldhaber and Kaplan 1975) and in the Florida Everglades (Bates et al. 1998) and reflects closed system behavior in the deeper parts of the system. That is, as sulfate reduction proceeds, the first sulfate reduced to sulfide is light, lea ving behind a relatively heavy residue in the process referred to as Rayleigh distillation. Therefore later (and deeper) formed sulfides are necessarily heavier because they derive their sulfur from this heavy residual pool. The trend to lighter sulfur in the surface sulfides with progression through the wetland indicates a decrease in sulfate reduction rate as the water moves through the wetland.

Mineralogically, the soils in the settling pond and cells 1 and 2 were similar. The X-ray diffraction analyses showed quartz to be the most abundant mineral. Also

Table 5. Within-cell variability of δ^{34}S in Cell 1. June 1999

Station/ depth		A	B	C	D
2	AVS	−20.76	−20.47	−26.68	−38.55
3	AVS	−4.12	−20.64	−23.18	−18.02
4	AVS	9.53	−28.01	−0.02	0.20
5	AVS	3.84		−4.30	−9.54
2	CrRS	−39.46	−19.72	−21.20	−38.25
3	CrRS	−23.46	−21.92	−23.22	−20.70
4	CrRS	−16.94	−18.71	−1.96	−12.24
5	CrRS	−32.14		−13.78	−17.13

Sample locations shown on Fig. 4

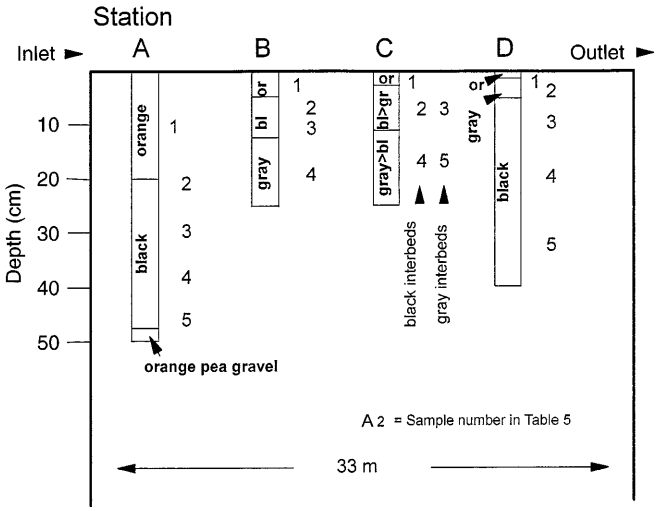

Fig. 5. Stratification of soil in cell 1. Each station has a surface oxidized layer, bright orange in color, underlain with a sharp transition by black or dark gray colored soil. the thickness of the orange layer decreases away from the inlet

present at each location were varying amounts of illite, kaolinite, goethite, native sulfur, gypsum, alunite, and gibbsite. All locations except cell 3 contained some alunite and gypsum, unusual minerals for wetland soils.

Cell 3 contained mostly quartz and varying amounts of illite, kaolinite, sulfur, goethite and gibbsite. Goethite was found in cell 3 only in the 3/97 trip. FeS and FeS_2 were not detected by XRD but measurements of total sulfur show that these should make up less than 1 or 2% of the minerals present and so would not be detected by this technique.

Applying the PHREEQC speciation/saturation program to the water chemical results from the wetland showed that a number of the minerals detected by x-ray diffraction are at or near saturation with the water. Figure 6 shows the water in each cell to be supersaturated with respect to the Al and Fe trihydroxides (gibbsite and amorphous $Fe(OH)_3$). The carbonate minerals of Ca, Mn, and Fe (calcite, rhodochrosite, and siderite) are at or very close to saturation in all analyses. Alunite, a K-Al sulfate, was found to be strongly supersaturated in the aeration pool and the settling pond and to be somewhat supersaturated in the cell 3 outlet, but strongly undersaturated in cell 1 water. The water in all samples was close to saturation with respect to the Ca sulfate, gypsum, but was always slightly undersaturated.

In April 1998, discharge was measured for the settling pond, cell 1, and cell 3. As expected, the values were very similar: 0.13 ft^3/sec (cfs), 0.14 cfs, and 0.11 cfs. The average for all cells was 0.12 cfs or 200 liters/minute. From these measurements and the cell volumes, the retention time for the settling pond was 1.5 days; for cell 1 and 2, 0.1 days; and for cell 3, 0.9 days. The whole wetland had a retention time of 2.6 days, probably a minimum value because a rainfall event immediately preceded the sampling.

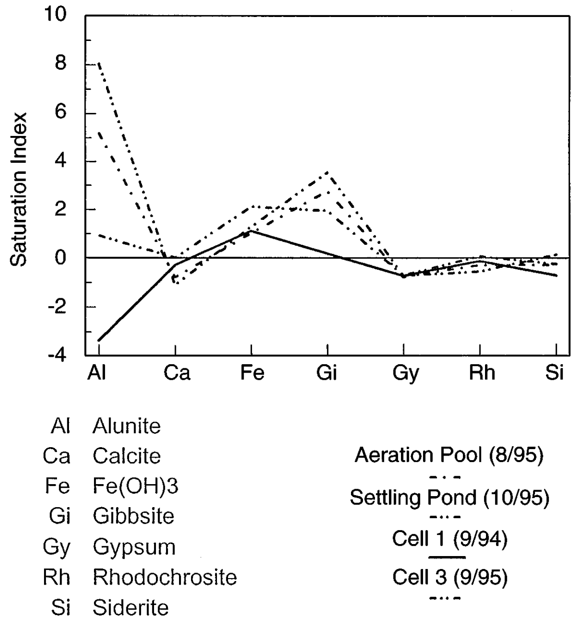

Fig. 6. PHREEQC results displaying saturation indices of mineral species for the Wills Creek wetland. The results suggest that equilibrium with alunite or gypsum will maintain a constant concentration of sulfate

4.4
Discussion

The Wills Creek wetland system was built as a series of compartments with different intended functions. The input is through anoxic limestone drains meant to establish a circum-neutral pH without precipitating iron oxides on the limestone. This function is working well, based on the relatively high pH of the water exiting the drain, a value that has tended to stay high throughout wetland history. Next in line (Fig. 3) is a settling pond to remove rapidly precipitated iron oxide, followed by two vegetated cells, 1 and 2, that were built to store metals (i.e. iron and manganese) in the soils and to keep them immobilized. Cell 3 was specifically designed to remove sulfate from the water by encouraging the reduction of sulfate into sulfide, therefore potentially immobilizing significant amounts of sulfide in the soils and increasing the alkalinity of the outlet water. Sulfate reduction by bacteria into sulfide was encouraged in cell 3 by promoting an anaerobic environment and providing a source of carbon, which is used as an electron donor by sulfate-reducing bacteria during net energy gain. An anaerobic environment was encouraged by establishing a subsurface flow in cell 3. A source of organic carbon was initially provided in cells 1–3 by the inoculation of organic substrates: mushroom compost in cell 1 and manures in cells 2 and 3.

Results from multiple measurements of influent and effluent water chemistry over seasonal sampling events during 1995–1999 at the Wills Creek wetland showed that cell 3 was not decreasing sulfate concentration levels. The wetland system as a whole produced some lowering of sulfate in 1994 and 1995, but from 1996 on, effluent sulfate exceeds influent sulfate. Sulfide was present, however, in the soils of all of the cells, so sulfate reduction is occurring to some degree. Sulfur isotopic measurements showed that sulfate reduction was too slow to have a significant remediation effect on the water passing through the wetland, in agreement with the water chemistry measurements. The sulfur isotope fractionation between the sulfide in the surface wetland soils and the aqueous sulfate was the greatest in cell 2, followed by cell 3 and cell 1. Because the amount of sulfur fractionation is inversely proportional to bacterial sulfate-reduction (Harrison and Thode 1958; Kaplan and Rittenberg 1964; Maynard 1980), the rate of sulfate reduction by bacteria was the fastest in cell 1, not in cell 3, contrary to the wetland design goals. The influent/effluent mass balance for the system and the sulfur isotopes of cell 1, the most active cell, both show that rates of sulfate reduction are declining. Moreover, pH values in the wetland were sharply lower in 1999 (Table 3), another indication of degrading performance. From the stable isotopes, we estimate reduction rates ranging from about 0.001 to 0.75 moles/liter × year. For comparison, McIntire and Edenborn (1990) reported values ranging from 0.0007 to 0.219 moles/liter × year for a wetland site in Pennsylvania.

It is interesting to consider what density of sampling is necessary to characterize wetland performance. For cell 1, if only station A2 samples are considered, average δ^{34}S of CrRS was –22.14 ± 9.67 permil (n = 5). If the remaining values for all stations B2, C2, D2 for all years are added, the average changes to –23.62 ± 5.73 (n = 10). Doubling of the sample size served mostly to decrease the uncertainty of the mean and not to change the value of the mean. We conclude that for rapid surveys, one sample per cell per season would likely be adequate, although it would be wise to spot check for within-cell variability initially.

The isotope results also indicate some seasonality to the performance of the wetland, especially cell 1, with greatly reduced activity in the cold season for the 1995–1997 time period. We attribute this seasonality to variation in bacterial activity with temperature. Other workers, for example Wildeman et al. (1990) and Wieder (1993), have reported a similar decline in wetland performance in the winter months. However, physical factors such as seasonal loading (Kepler 1990; Stark et al. 1990) or water levels (Hedin and Nairn 1990) have been cited as the causes of seasonal variation in performance. Our survey of isotopes with depth is consistent with either a temperature effect or a recycling of deeper sulfur into the shallower layers during the summer perhaps resulting from changes in water levels.

Sulfate concentration levels were not decreasing with time or as the water moved through the wetland for the 1995–1999 time period. Therefore, although sulfate reduction was occurring, it was having no effect on dissolved sulfate concentrations. PHREEQC results showed that two sulfate minerals, alunite and gypsum, were supersaturated or very close to saturation in the water of all of the cells, and x-ray diffraction showed that these two minerals were in fact present in the soils of cells 1 and 2. The importance of gypsum and alunite in the Wills Creek wetland is that they buffered the calcium and sulfate concentrations in the water. According to Langmuir (1997), the most common mineral phases found near or at saturation in acid mine waters are ferric oxyhydroxides and gypsum. Figure 7 shows that the Wills Creek wetland was at its concentration limit of calcium and sulfate. Bacterial sulfate reduction was occurring in cells 1–3 as evidenced by blackened sediment and the sulfur isotope fractionation between the sulfate and sulfide (pyrite). However, an oxidized layer containing mainly iron oxides, calcium sulfates, and aluminum sulfates coated the top of the substrate (Figure 5). This layer effectively isolates the bulk of the wetland soil from the overlying water, and the sulfate-reducing bacteria use the sulfate from the gypsum and alunite in the surface oxidized layer, not the sulfate in the water in their metabolism. Therefore, the bacteria are not affecting the sulfate concentration levels in the water.

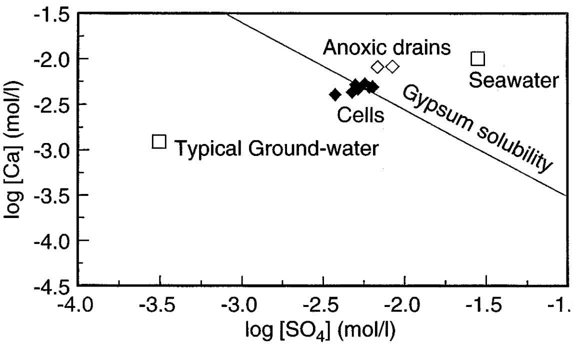

Fig. 7. Plot of log $[\mathrm{Ca}^{2+}]$ versus log $[\mathrm{SO}_4^{2-}]$ for 12 water samples from the anoxic drain, aeration pools, cell 1, and cell 3 in 1994 showing a close approach to equilibrium with gypsum for most samples

The wetland does not have a large enough surface area to accommodate the sulfate load, so the sulfate-reducing bacteria cannot completely remove the mineral sulfate in the oxidized layer so that gypsum and alunite are able to remain in equilibrium and buffer the sulfate concentration of the overlying water.

For the period 1995–1997, the amount of fractionation was least in cell 1, intermediate in cell 3, and greatest in cell 2, indicating that the rate of sulfate reduction was fastest in cell 1 followed by 3 and then 2. This sequence of reduction rates does not correlate to vegetation or to style of flow (surface versus subsurface). The factor that does correlate is the original organic substrate. Cell 1 had spent mushroom compost, cell 3 had Fermway, a trademarked product composed of a mixture of fermented chicken and cow manure, and cell 2 had rotted cow manure. The mushroom compost cell showed the greatest initial bacterial activity, which agrees with other studies showing this to be the most effective organic substrate for acid-drainage wetlands (Wieder 1993). Thus we have the rather surprising result that, as far as sulfate reduction is concerned, wetland performance is relatively insensitive to configuration and to flow patterns. The nature of the organic matter in the wetlands dominates for sulfate reduction and is likely to do so for other bacterially-mediated reactions. The convergence of reduction rates in cells 1 and 2 during the 1999 sampling event most likely reflects the depletion of the original organic matter and the dominance of *Typha* leaves as the new organic substrate in both cells.

5
Conclusions

5.1
Behavior of the wills creek wetland

Some aspects of the performance of the Wills Creek wetland in remediating acid mine drainage agree with predictions from the literature, but some are at variance:

- The anoxic limestone drains performed well and showed no signs of deterioration in performance over the period of study.
- Mushroom compost is a much better organic substrate than manures for encouraging bacterial processes like sulfate reduction in wetlands, although this advantage decreases with time as *Typha* detritus begins to dominate the organic matter.
- Staged wetlands with different types of cells are no more effective than a single wetland style based on surface flow with baffles. The construction of the third cell in the Wills Creek design appears to have been a waste of resources.
- Sulfur isotopic fractionation can be seasonal, so that removal processes that depend on bacterial activity appear to lag in the winter months, impairing the wetland's remediation ability. It is not clear how much of this seasonality is caused by changes in bacterial growth rates and how much is recycling of heavier sulfide from deeper levels during the summer months.

5.2
Policy implications

These observations about the Wills Creek wetland in turn suggest some general policies in designing and evaluating wetlands intended for remediation of mine drainage:

- Removal of acidity and iron can be accomplished by using anoxic limestone drains followed by modest-sized surface-flow wetlands whose initial organic substrate is spent mushroom compost. Significant sulfate removal by bacterial sulfate reduction, however, requires cells with a larger surface area than is predicted from the sizing criteria based on iron removal.
- The efficiency of a constructed wetland or an individual cell in a wetland can be rapidly and effectively monitored by measuring the sulfur isotope spread between the sulfide in the soil and the sulfate in the water. This technique provides an integrated picture of wetland performance over time in contrast to the snapshot view provided by water chemistry alone. Also, this approach permits evaluation of the performance of individual wetland cells and even portions of cells, which is not possible from the record of input/output water chemistry.

The seasonality of wetland performance suggests that they are ideally suited to remediation of pollution problems in tropical countries. The low capital cost and simple technology are additional factors that suggest that well-designed wetlands could become an important remediation strategy for environmental problems in third-world countries, although they are as yet not widely used (Denny 1997).

6
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