Manganese removal by chemical and microbial oxidation and the effect on benthic macroinvertebrates at a coal mine in Wayne County, western West Virginia

by E.I. Robbins[1]
R.R. Maggar[2]
E.J. Kirk[3]
H.E. Belkin[1]
H.T. Evans, Jr.[1]

([1]U.S. Geological Survey, National Center MS 956, Reston, VA 20192)
([2]Pen Coal Corporation., Dunlow, WV)
([3]REIC Laboratory, Inc, Beaver, WV)

ABSTRACT

Manganese removal from discharge waters is a serious problem in many states where Appalachian Basin coal is mined. We analyzed benthic macroinvertebrates and bacteria upstream and downstream from mining operations at the Queens Fork surface mining operations in Wayne County, western West Virginia, in order to assess the ecological effect of elevated instream Mn levels. Manganese data were also retrieved from the U.S. Geological Survey QWDATA database to compare mine wastewater Mn concentrations with those in other Wayne County surface and ground waters. The benthic macroinvertebrates included sensitive, facultative, and tolerant species. A small shift from more sensitive types upstream to more tolerant ones downstream is occurring throughout the ongoing benthic study. No statistically significant differences in abundance and number of taxa existed between the upstream and downstream localities. Rocks upstream and downstream from mining are coated black from Mn oxide precipitates. Glass microscope slides were placed in various sites to mimic rock surfaces and allow bacteria to colonize them for study. The major microbial taxon precipitating Mn upstream and downstream from mining was Leptothrix discophra, a species that is typical of natural settings having elevated Mn levels. Other unidentified Mn-fixing species dominated within the wastewater treatment ponds. Dissolved Mn concentrations in wells are higher than surface waters suggesting that many streams in Wayne County are fed by ground waters carrying elevated concentrations of Mn.

INTRODUCTION

Manganese is one of the most difficult elements to remove from surface waters (10, 22, 3 1).

Although dissolved Mn is not known to be toxic, and even blocks the toxic effect of H' (7), it has undesirable effects on water use. These include staining laundry and ceramic fixtures such as toilets where concentrations are greater than 0.05 mg/L (14). Federal regulations therefore control discharge limits. In drinking water sources, the secondary maximum contaminant level (SMCL) for Mn must not exceed 0. 05 mg/L (28). Acid-generating mines may discharge a maximum allowable 4 mg/L as long as the average of daily values for 30 consecutive discharge days does not exceed 2 mg/L (26). Discharges are also regulated by individual states. West Virginia's instream water quality limit is 1.0 mg/L (32).

Many studies have been conducted on Mn removal in the past, including those designed to evaluate chemical dynamics, experiment with packed columns of limestone, and evaluate passive treatment systems (2, 5, 8). Microbial remediation efforts include designed wetlands, microbial bioreactors, and pellets of mixed microbial cultures (1, 6, 29, 30).

In West Virginia, anomalous Mn occurs in a variety of geological settings. Southeastern West Virginia hosts strategic deposits of Mn in Lower Paleozoic rocks (11) (Fig. 1). Stream concentrate samples in eastern and northern parts of the state pinpoint local Mn concentrations (9). Mining operations in many Pennsylvanian age coals in the Appalachian Basin discharge Mn, along with a large variety of other major and trace elements (13, 15, 21).

Manganese removal is also a problem during coal mining in Wayne County, which is in western West Virginia. Pen Coal Corp. mines the 5-Block coal (Lower Kittanning-equivalent) along Kiah Creek (Fig. 1). Mn is concentrated in the sandstone overlying the coal in some areas of the operation (Fig. 2), similar to a situation that has been analyzed in Pennsylvania (20). Mine discharge at several localities is acidic, in the pH 2-3 range. Discharge is treated to raise alkalinity and precipitate Mn (Fig. 3).

Two studies were undertaken to learn about natural instrea m processes in the vicinity of coal mining along Kiah Creek. A long term benthic macroinvertebrate study was begun in 1995 to assess the ecological nature of the effect of elevated Mn levels upstream and downstream of mining operations. A 6-week-long microbial study was undertaken to learn the nature of the indigenous and adapted bacteria upstream and downstream of mining operations.

MATERIALS AND METHODS

Study site: The study site lies near Kiahsville in Wayne County, WV (Fig. 1). The Queens Fork surface mining activities discharge into Kiah Creek. Kiah Creek is a tributary of the East Fork of Twelvepole Creek which flows into East Lynn Lake, and then into the Ohio River; East Lynn Lake is regulated by the Army Corps of Engineers. Discharge moves through a series of treatment ponds (Fig. 3). At the Pond 2 locality, mine discharge is directly treated at the inflow with CaO and NaOH and then enters Pond 2. Flocculates settle behind a barrier and clear water is discharged to Pond 2A. Additional NaOH is added at this point to elevate the pH to 11 to precipitate the remaining Mn to meet effluent limits. The outflow of Pond 2A discharges across large rocks and then into Kiah Creek. At the Pond 3 locality, mine discharge is treated at the inflow first with CaO and then with CaO and NaOH. The flocculates in Ponds 2A and 3A are periodically pumped up to drying cells and then permanently disposed.

Sites were chosen upstream (control) and downstream from these discharge points for benthic, macroinvertebrate and microbial analyses. The invertebrate studies were conducted on Kiah Creek just below its confluence with Witcher Fork (upstream control) and downstream from mining operations at Queens Fork (Fig. 1). Microbial studies were undertaken on samples collected from mine discharge, within treatment ponds, at pond discharges, downstream from ponds, as well as in Parker Branch upstream from mining operations (Fig. 1).

A sandstone sample was collected from the backfill of the Queens Fork surface mines for laboratory microbial experiments. The gray and brown sandstone overlies the 5-Block coal and contains thin lenses of coal and streaks of black/brown Mn oxide.

<u>Benthic macroinvertebrates:</u> Standard kick-net and Surber samplers (EPA's Rapid Bioassessment Protocol) were used in both upstream and downstream localities to collect benthic. macroinvertebrates (aquatic insects, molluscs, and worms). Insects were identified to lowest practical taxonomic level, and trophic relationships and pollution sensitivities were assessed using standard methods (17).

<u>Rock chemistry:</u> Chemical analyses of materials disturbed by mining was undertaken to locate the sources of easily soluble Mn so as to be able to avoid them or handle them specially during mining. Paste pH, net neutralization potential, total Mn, and easily leachable Mn were analyzed according to standard procedures (3). Details of treatment are discussed in Maggard (this volume).

<u>Water chemistry:</u> Water samples were collected from upstream and downstream localities during benthic macroinvertebrate studies and measured on site for water temperature, pH, conductivity, and dissolved oxygen (DO). Water samples were appropriately preserved and returned to the lab for further analysis. Water chemistry was analyzed using current EPA-approved analytical methodology.

Manganese data for surface and wells in Wayne County were retrieved from the USGS water-quality database (QWDATA). The data were analyzed with the STATIT statistical package.

<u>Microbial **analysis:**</u> Three types of samples were collected for analysis by light microscopy:

(1) Flocculates and precipitates collected in the field (May 7, 1996) were analyzed. Motile (moving) and non-motile bacteria, cyanobacteria, algae, fungi, protozoans, and other organic matter were noted.

(2) Glass microscope slide sets were left at various locations) upstream and downstream of mining operations to learn about the benthic microbial community (see 18 and 24 for techniques). Multiple slide sets were left for 6 weeks (May and June 1996). After retrieval, the slides were analyzed for colorless, iron-encrusted, and manganese-encrusted microorganisms that settled on the slides. The presence of oxidized Mn was tested with o-Tolidine (19).

(3) In the laboratory, a crushed sample of Mn oxide-coated sandstone was placed along with a sterile glass slide set in a loosely-covered beaker filled with sterile deionized water to allow indigenous bacteria from the sandstone to settle on the slides. The slides were retrieved after 3 months and the benthic microbial community was analyzed.

<u>Microbial statistics.</u> The number of holdfasts, which are attachment structures of the

bacterium Leptothrix discophora, were counted in equal areas on replicate slides left upstream and downstream from mining.

Mineralogy: Two slides that were particularly heavily coated with Mn from the final discharge water were subjected to X-ray powder diffractometry (Debye-Scherrer camera). This technique uses a few micrograms of powdered sample.

SEM: Selected slides were analyzed by scanning electron microscopy (SEM) using a JEOL JSM-840 scanning microscope equipped with a Princeton Gamma Tech X-ray energy dispersive analyzer (EDAX). (The use of brand name does not imply endorsement by the USGS.)

RESULTS

Studies conducted upstream and downstream from mining operations found similarities and dissimilarities in all parameters.

Benthic macroinvertebrates. Benthic macroinvertebrate communities were analyzed according to their abundance, taxa. diversity, and tolerance to pollution (Table 1). The presence of pollution sensitive individuals is important for the purposes of understanding the potential role of Mn in the environment. By combining the three upstream sampling dates and comparing them to the three combined downstream dates, the two sites appear relatively similar. When upstream dates are combined, a total of 1, 148 aquatic insects (29 taxa) were collected. Of this, 16.9 % of the abundance were pollution sensitive (11 taxa); 14.4 % were pollution facultative (9 taxa); and 68.8 % were pollution tolerant (9 taxa). When downstream dates are combined, a total of 1,478 aquatic insects (26 taxa) were collected. Of this 13.9 % were pollution sensitive (10 taxa); 7.6 % were pollution facultative (9 taxa); and 78.4 % were pollution tolerant (7 taxa).

Rock chemisry. The sources of easily soluble Mn were located (Fig. 2). Coarse-grained sandstone in proximity to coal and deficient in neutralization potential contained the most soluble Mn. A strong correlation between potential acidity, which is calculated from total sulfur content, and easily soluble Mn was noted (see 16).

Water chemistry. Some generalizations can be drawn from the upstream and downstream water quality data for the April and October collection dates (Table 2). Seasonal variations were evident. When flow was low, there were significant increases in the concentrations of Ca, SO, Na, Fe, and Mg in the downstream direction. When flow was high, Na increased slightly; TDS, hardness, alkalinity, SO, Ca, and Mg increased by a factor of 2; and Mn increased by a factor of 10. Temperatures were lower downstream than upstream.

A statistical analysis of Mn data for streams and wells in Wayne Co. revealed some interesting trends (Table 3). Total Mn in streams ranged from < 0. 0 10 to 2 mg/L, and dissolved Mn ranged from < 0. 00 1 to 2 mg/L. Dissolved Mn in wells ranged from < 0. 00 1 to 1. 4 mg/L. A comparison of mean and median concentrations shows that total Mn is twice as high as dissolved Mn in streams of Wayne County. The average (mean) concentration of dissolved Mn in 86 wells in Wayne County is higher than that of streams.

Macroscopic observations of use to microbial analyses. Dependant on pH, the products of discharge, of chemical fixation by NaOH and CaO (Fig. 3), and of microbial precipitation are a colorful mixture of black Mn-rich, red Fe-rich, and white At-rich phases. Outflow from one Queens Fork mine is at a rock shelter that is coated red by thick iron-oxide precipitates. At the treatment pond discharges, rocks are coated black from Mn oxide. At the Parker Branch control site, rocks in the riffles are also coated black.

Microbial analysis. Numerous types of bacteria, along with cyanobacteria, algae, fungi, and protozoans settled on glass slides left in the upstream control site, at the mine outflow site, in the treatment ponds, downstream from the treatment ponds, and in the sandstone laboratory experiment (Table 4). Colorless, iron-coated, and Mn-coated cocci, rods (rod-shaped bacteria), and filaments were present on all slides (Figs. 4, 5 and 6). Diatoms were abundant on slides from each environment with the exception of the acid discharge from the mine and at the pipe that discharges NaOH.

Manganese oxide precipitates occurred as dark brown or black stains on the slides. The role of bacteria in Mn oxidation was evident by a wide variety of precipitates that were intimately associated with the bacteria. Brown films were laced with numerous colorless rods (Figs. 4,4, 4,8, and 5,17). Filamentous sheaths were brown (Figs. 4,7 and 5,20) or were becoming coated (Figs. 5,13 and 5,14). Individual rod-shaped bacteria were brown (Fig. 6,23). Specific bacteria having identifiable structures included clumps that resembled Siderocystis sp. (Fig. 4,5). The most distinct structures were the typical spherical or doughnut-shaped holdfasts of Leptothrix discophora that were collected upstream and downstream of mining operations, as well as at discharge locations (Figs. 4,9-12 and 5,18-19). These localities also had something quite unusual -- some of the holdfasts were intricately elaborate structures that radiated out from individual holdfasts (Fig. 4,6).

The Mn-precipitating microbial populations upstream predominantly formed holdfasts and brown-coated rods. Downstream, the population was much more varied and diatoms were extremely abundant. Downstream, Mn was also precipitated by coated filaments and cf. Siderocystis sp. Brown amorphous particles and brown films colonized by short rods were also present.

The milky brown flocculate in the treatment ponds, in contrast, was obviously a chemical precipitate of a paste-like substance (Fig. 4,2). The highly alkaline treatment ponds were not sterile environments, however. Instead, bacteria, cyanobacteria, algae, fungi, and protozoans lived in them (Table 5).

Microbial statistics. The average number of L. discophor a holdfasts on slides left upstream was 1, 130 holdfasts/cm. The average of downstream counts was 1, 100 holdfasts/cm^2. If the colonization process were linear, then the average colonization rate upstream and downstream would be 28 holdfasts/cm^2/day.

Mineralogy. Although the black and brown precipitates were granular and rarely birefringent using petrographic microscopy, the material was poorly crystallized and produced 6 diffuse lines by X-ray powder diffractometry. The mineral(s) that produced these lines could not be matched by any single phase listed in the Joint Powder Diffraction Data Commission.

SEM. Mn was concentrated in discrete areas on and around holdfasts (Fig. 6), but not on any analyzed filament. Mn, Ca, and S were the only elements present at the limit of analytical detection, along with Si from the diatoms.

Sandstone experiment . L eptothrix discophora holdfasts attached to glass slides in the laboratory sandstone experiment (Fig. 4,12). The average count on the slides was 40 holdfasts/cm^2.

INTERPRETATIONS AND CONCLUSIONS

Although we have focused this paper on the chemical and microbial processes that remove Mn and the effects of elevated Mn on invertebrate communities, it is not possible to control chemical, physical, or biological variables in the field. Obviously, Mn is only one of a variety of potential controlling variables that govern the distribution of organisms in the streams.

The benthic macroinvertebrates included sensitive, facultative, and tolerant species. Among the sensitive insects, there were individuals that were found strictly upstream from mining operations and others that were found strictly downstream. Only a small shift from more sensitive types upstream to more tolerant ones downstream occurred throughout the ongoing benthic study. There were no statistically significant differences between the upstream and downstream sites for both abundance and number of taxa. For this reason, one can assume that negative impacts from mining have been kept to a minimum.

Analyses of water quality parameters showed that most increased in the downstream direction. Chemical analyses showed that downstream habitats are subjected to more SO,, Ca, Mg, and Mn than in upstream habitats. The concentrations vary with flow rates.

The statistical analysis of Mn data for streams and wells in Wayne County suggests the presence of dynamic trends in interactions among surface water, bed sediments, and ground water. Total Mn was twice as high as dissolved Mn in streams, which suggests a large component of Mn transported in streams is suspended rather than dissolved. Bed sediments, particularly rocks that are coated black, contain a large flux of Mn that could be transported during high streamflow events. Because the mean concentration of dissolved Mn in well water was higher than in streams, a relatively large proportion of Mn in Wayne County streams may be derived from Mn in ground waters that discharge to the streams. For both ground water and surface water in Wayne County, average concentrations of Mn exceed the U.S. EPA SMCL of 0. 05 mg/L. A total of 152 of 268 streams (5 6.7 %) analyzed for dissolved Mn had concentrations greater than or equal to the 0.05 mg/L SMCL for drinking water. Based upon the data available from the USGS water-quality database (QWDATA), only 6 of 268 sites (2.2 %) analyzed for dissolved Mn and only 4 of 187 sites (2.1 %) analyzed for total Mn had concentrations greater than the I mg/L West Virginia State standard for streams.

The microbial populations of Mn-rich flocculates are quite varied. The bacteria that fix or oxidize Mn onto solid glass surfaces, which are used to mimic rocks in riffles, form simple brown rods and simple Leptothr ix discophora holdfasts, as well as highly complex and elaborate holdfast structures. The simple holdfasts are structures that the rod-shaped bacteria use to attach to surfaces and are typical of those precipitating Mn in streams having elevated Mn concentrations elsewhere (4, 23, 24, 25). The elaborate holdfast structures are unusual and occur both upstream of mine operations and in mine treatment ponds. The highly efficient Metallogenium -type Mn-fixing bacteria isolated by Vail and others (1988) inorganic-matter-rich environments were not found. Lacking these, the thin coatings of Mn on rocks in swiftly moving water show that rocks serve, at the very least, as mildly efficient Mn-stripping structures. Rates of Mn removal by coatings on rocks need to be addressed in further studies.

Holdfasts of Leptothrix discophora also attached to glass slides in the 3-month-long laboratory sandstone experiment. These bacteria typically attach where anoxic, Mn-bearing ground water is present; they then proceed to oxidize the Mn on their holdfasts where water is oxygenated (23). Therefore, the sandstone Mn was probably in a reduced form and the deionized water of the experiment supplied oxygen. The results were not unexpected because this bacterium will colonize new surfaces as they become available near redox boundaries in beakers or test tubes filled with natural waters and placed in the laboratory (Robbins, pers. obs.). The fact that no organic matter was added to the sterile beaker water suggests either the bacterias' organic compound requirements are minimal or this strain may be able to oxidize Mn as an energy source, therefore being autotrophic in its nutrition.

This information about the different types of bacteria that precipitated oxidized Mn, and the different forms that the precipitates took, are very useful for explaining how Mn is bound in mine treatment situations. Evangelou and others (1992) recognized that Mn removal is catalyzed at mineral surfaces; the bacteria that participate in the catalysis in this West Virginia locality are dominated by L. discophora . Watzlaf (1988) stressed adsorption onto reactive surfaces as an important process in mine drainage treatment. In reality, these bacteria precipitate Mn on a wide variety of surfaces including non-reactive ones such as glass slides.

The current effluent limits for Mn in receiving streams that are not used for domestic purposes have been shown to be lower than can be supported by scientific research (7, 12, 27). Our analyses show that benthic macroinvertebrates do not appear to be affected in any significant manner by the levels of Mn in the control stream or in the mine discharge. Furthermore, it appears that the microbial population proliferates to take advantage of the Mn concentration in the water.

ACKNOWLEDGEMENTS

We would like to thank Mark Kazoo (USGS-WRY-WV) for retrieval and initial interpretation of Mn concentrations in Wayne County waters, and Bob Kleinmann for useful editorial comments.

REFERENCES

1. Division, Jo, 1991, Bio-crab and wetlands - Passive affordable acid mine drainage treatment: Proceedings, 12th Ann. West Virginia Surface Mine Drainage Task Force Symposium, Morgantown, WV 11 P.
2. Evangelou, V.P., Sainju, U.M., and Huang, Xiao, 1992, Evaluation and quantification of armoring mechanisms of calcite, dolomite, and rock phosphate by manganese, in Younos, Tamim., Diplas, P., and Mostaghimi, S., eds., Land Reclamation; Advances in Research and Technology: St. Joseph, MI, American Society of Agricultural Engineers, Publication 14-92, p. 304-316.
3. Evans, Phil, and Skousen, Jeff, 1995, Effect of digestion method on neutralization potential of overburden samples containing siderite, in Skousen, J.G., and Ziemkiewicz, eds., Acid Mine Drainage Control and Treatment: Morgantown, WV, West Virginia

University and National Mine Land Reclamation Center, p. 21-33.
4. Ghiorse, W.C., 1984, Bacterial transformation of manganese in wetland environments, in Klug, M.J., and Reddy, C.A., eds., Current Perspectives in Microbial Ecology: Washington, DC, American Society of Microbiologists, p. 615-622.
5. Gordon, J.A., and Burr, J.L., 1987, Manganese treatment by two methods at abandoned coal sites in North Alabama, in Proceedings, National Symposium on Mining, Hydrology, Sedimentology, and Reclamation: Lexington, KY, University of Kentucky College of Engineering, p. 383-393.
6. Gordon, J.A., and Chuang, N.S., 1990, Biological treatment of manganese from mining seepage: Pilot plant studies, in Proceedings of the 44th Purdue Industrial Waste Conference: Boca Raton, FL, CRC Press, p. 299-305.
7. Grippo, R. S., and Dunson, W.A., 1996, The body ion loss biomarker. 1. Interactions between trace metals and low pH in reconstituted coal mine-polluted water: Environmental Toxicology and Chemistry, v. 15, p. 1955-1963.
8. Hedin, R.S., Nairn, R.W., and Kleinmann, R.L.P., 1994, Passive treatment of coal mine drainage: Bureau of Mines Information Circular 9389, 35 p.
9. Hinkle, M.E., Watts K.C., and Griffitts, W.R., 1994, Isopleth maps of iron, manganese, vanadium, and related elements in stream sediments of West Virginia: U.S. Geological Survey Map I 2364-D.
10. Jordan, C.F., and Phillips, T.D., 1981, The removal of iron and manganese from mine water effluents: Symposium on Surface Mining Hydrology, Sedimentology and Reclamation, University of Kentucky, Lexington, KY, p. 247-249.
11. King, H. M., and Kirstein, D. S., 1989, Manganese in the mountain state: Mountain State Geology 1989, p. 18-21.
12. Kleininann, R.L.P., and Watzlaf, G.R., 1988, Should the effluent limits for Mn be modified?: Bureau of Mines Information Circular 9183, Proceedings of the Mine Drainage and Surface Mining Restoration Conference, Pittsburgh, PA, p. 305-310.
13. Knapp, N.F., 1977, Trace elements in Ohio coals: Ohio Division of Geological Survey Report of Investigations 103, 12 p.
14. Kozar, M.D., and Brown, D.P., 1995, Location and site characteristics of the ambient ground water quality monitoring network in West Virginia: U.S. Geological Survey Open-file Report 95-0130, 48 p.
15. Lindgren, L. C., 1985, Trace elements in Kentucky No. 9 coal and their implications, in Cross, A.T., ed., Economic Geology,; Coal, Oil and Gas: Comptes Rendu, International Congress on Carboniferous Stratigraphy and Geology, v. 9, p. 377-386.
16. Maggard, R.R., Manganese contaminated discharges: Treatment and prevention, this volume.
17. Merritt, R.W., and Cummins, K.W., 1984, An Introduction to the Aquatic Insects of North America, 2nd ed.: Dubuque, IA, Kendall/Hunt, 722 p.
18. Mills, A.L., and Lawrence, Mallory, 1987, The community structure of sessile heterotrophic bacteria stressed by acid mine drainage: Microbial Ecology, v. 14, p. 219-232.
19. Morgan, J.J., and Stumni, Werner, 1965, Analytical chemistry of aqueous manganese: Journal of the American Water Works Association, v. 57, p. 107-119.
20. Morrison, J., Atkinson, S., and Scheetz, B., 1990, Delineation of potential manganese sources in the coal overburdens of western Pennsylvania, in Proceedings of the 1990 Mining and Reclamation Conference: Morgantown, WV, West Virginia University Publication Service, p. 249-256.

21. Plass, W.T., 1976, Changes in water chemistry resulting from surface mining of coal on four West Virginia watersheds: Green Lands Quarterly, v. 6, p. 22-27.
22. Richards, R.P., and Foellmi, S.N., 1985, Utility battles iron and manganese: Public Works, v. 116, no. 6, p. 91-95.
23. Robbins, E.I., D'Agostino, J.P., Ostwald, J., Fanning, D.S., Carter, Virginia, and Van Hoven, R., 1992, Manganese nodules and microbial oxidation of manganese in the Huntley Meadows wetland, Virginia, USA: Catena. Supplement (Dutch Soils Jour.), v. 21, p. 1-23.
24. Robbins, E.I., and Hayes, M.A., 1996, What's the red in the water? What's the black on the rocks? What's the oil on the surface? http://pubs.usgs.gov/publications/text/norriemicrobes.html
25. Robbins, E.I., and Norden, AW., 1994, Microbial oxidation of iron and manganese in wetlands and creeks of Maryland, Virginia, Delaware, and Washington, D.C., in Chiang, S. -H., ed., 11 th Annual International Pittsburgh Coal Conference, Proceedings, Vol. 2, p. 1154-1159.
26. U.S. Code of Federal Regulations, 1996, Title 40--Protection of the environment. Chapter 1-EPA, Part 434-Coal mining point source categories, Subpart C--Acid or ferruginous mine drainage: Washington, DC., U.S. Environmental Protection Agency, p. 222-223.
27. U.S. Environmental Protection Agency, 1986, Quality criteria for water: EPA 440/5-86-001, Office of Water Regulations and Standards, Washington, DC.
28. U.S. Environmental Protection Agency, 1994, EPA drinking water regulations and health advisories: Office of Water 4301, EPA 822-R-94-003, 11 p.
29. Vail, W.J., and Riley, R.K., 1995, U.S. Patent 5,441,641, Process for removing manganese from solutions including aqueous industrial waste: U.S. Patent Office, 2 p.
30. Vail, W.J., Wilson, S, and Riley, R.K., 1988, Mine water and mine waste; Isolation and culture of manganese-oxidizing bacterium from a man-made cattail wetland: Bureau Mines IC 9183, Proceedings of the Mine Drainage and Surface Mining Restoration Conference, Pittsburgh, PA, USA, April 17-22, p. 399.
31. Watzlaf, G. R., 1988, Chemical stability of manganese and other metals in acid mine drainage sludge: Bureau of Mines Information Circular 9183, Proceedings of the Mine Drainage and Surface Mining Restoration Conference, Pittsburgh, PA, USA, April 17-22, p. 83-90.
32. West Virginia Water Resources Board, 1993, Title 46. Legislative Rules, State Water Resources Board, Requirements Governing Water Quality Standards, Series 1, Appendix E-Specific Water Quality Criteria, 13 p.

Table 1. Benthic collection data for localities upstream and downstream of mining operations along Kiah Creek. (Classification of pollution indicator organisms: F = facultatitve, S = sensitive, T = tolerant, U = unclassified) (*Not included in calculations)

Benthic macroinvertebrates	Downstream			Upstream			Trophic Relationships
	4/27/95	4/19/96	10/6/96	4/27/95	4/19/96	10/6/96	
INSECTA							
Coleoptera (Beetles)							
Psphenidae undiff. (S)	--	--	1	1	--	1	scraper
Elmidae undiff. (S)	--	1	62	--	22	23	gatherer/scraper
Diptera (True flies)							
Tipulidae undiff. (T)	1	8	22	--	15	25	--
Tipula sp. (T)	--	1	--	--	--	--	shredder/gatherer
Antocha sp. (T)	--	24	--	--	223	--	gatherer
Dicranota sp. (T)	--	1	--	1	--	--	predator
Ceratopogonidae undiff. (F)	18	1	4	3	4	--	predator/gatherer
Simuliidae undiff. (F)	--	--	2	--	--	8	filterer
Chironomidae undiff. (T)	156	464	22	88	350	33	gatherer/filterer/ shredder/miner
Empididae undiff. (T)	--	--	--	3	--	--	predator/gatherer
Ephydridae undiff. (T)	--	--	1	--	--	--	gatherer/shredder
Ephemeroptera (Mayflies)							
Ameletus sp. (S)	--	--	--	--	4	--	gatherer
Isonychia sp. (F)	--	--	1	--	--	4	filterer
Baetis sp. (F)	2	4	--	4	2	4	gatherer/scraper
Stenacron sp. (S)	13	1	29	17	2	18	gatherer/scraper
Stenonema sp. (S)	5	16	16	7	4	8	gatherer/scraper
Ephemerella sp. (F)	4	19	1	4	13	1	gatherer/scraper
Caenis sp. (F)	38	--	--	6	24	12	gatherer/scraper
Baetisca sp. (S)	4	15	17	36	21	24	gatherer/scraper
Leptophlebiidae undiff. (S)	--	1	--	--	--	--	gatherer/scraper
Ephemera sp. (F)	46	--	2	--	--	--	gatherer/predator
Hexagenia sp. (F)	--	1	--	--	--	--	gatherer
Eurylophella sp. (F)	--	--	--	--	1	--	--
Megaloptera (Hellgrammites)							
Corydalidae undiff. (S)	--	--	--	2	--	--	predator
Corydalus sp. (S)	--	--	1	--	--	1	predator
Odonata: Anisoptera (Dragonflies)							
Gomphidae undiff. (T)	1	--	1	1	--	1	predator
Aeshnidae undiff. (T)	--	--	1	--	--	1	predator
Odonata: Zygoptera undiff. (Damselflies)							
Coenagrionidae undiff. (T)	1	--	--	--	--	--	predator
Plecoptera (Stoneflies)							
Amphinemura sp. (F)	--	--	--	--	8	--	gatherer
Leuctra sp. (F)	--	4	--	--	--	--	shredder
Isoperla sp. (S)	2	--	--	--	--	--	predator/gatherer
Chloroperlidae undiff. (S)	2	4	--	--	--	--	predator/gatherer
Trichoptera (Caddisflies)							
Polycentropodidae undiff. (S)	--	--	--	1	--	--	gatherer/predator
Polycentropus sp. (S)	--	--	1	--	--	--	predator/gatherer
Hydropsychidae undiff. (F)	4	--	20	1	10	18	filterer
MOLLUSCA							
Decapoda (Crayfish)							
Astacidae undiff. (crayfish) (S)	1	--	--	3	--	--	gatherer
undiff.* (U)	--	--	--	--	--	1	gatherer
Pelecypoda (Clams)							
fingernail clams* (U)	--	--	--	--	--	1	filterer
ANNELIDA							
Oligochaeta (worms) (T)	36	11	34	40	155	223	--
Total number of individuals	336	576	235	215	858	405	
Total number of taxa	19	16	16	17	15	17	
% Sensitive individuals	8.1	6.6	55	31.6	6.2	21	
% Faculative individuals	33.5	5.0	10	6.5	7.2	9	
% Tolerant individuals	58.4	88.4	35	61.8	86.6	70	
% Unclassified individuals	0.0	0.0	0	0.0	0.0	0	
% Total	100.0	100.0	100	99.9	100.0	100	

Table 2. Water quality variables in Kiah Creek upstream and downstream from mining operations

PARAMETER	Upstream				Downstream			
	4/27/95	10/27/95	4/19/96	10/6/96	4/27/95	10/27/95	4/19/96	10/6/96
Flow (ft^3/s)	84.87	0.8	10.5	12.8	162.72	2.7	11.4	18.2
Temperature (°C)	15.5	11.2	15.2	13.9	13.4	11.0	12.5	11.1
Dissolved O_2 (mg/L)	8.95	10.02	9.05	9.06	9.06	9.54	9.30	9.61
pH (pH units)	7.29	7.43	7.69	6.79	7.53	7.00	7.69	7.50
Conductance (μmhos)	113.6	613	196.0	233.0	193.8	782	334.0	316.0
TDS (mg/L)	74.0	404	115.0	111.0	121.0	551	205.0	158.0
TSS (mg/L)	10.0	33	4.0	<1.0	12.0	10	5.0	1.0
Hardness (mg/L)	36.9	265	67.6	46.6	72.3	368	138.0	82.9
Alkalinity (mg/L)	9.1	55.5	13.8	15.9	19.4	65.7	27.2	25.5
Acidity (mg/L)	21.6	nd	nd	<1.0	<1.0	nd	nd	<1.0
SO4 (mg/L)	24.4	188	46.9	68.8	54.3	301	102.0	106.0
Na (mg/L)	3.03	18.4	3.22	4.74	3.30	18.5	3.58	5.26
Al (mg/L)	0.39	0.22	0.20	<0.10	0.47	0.22	0.25	<0.10
Ca (mg/L)	9.28	58.1	13.5	18.0	19.5	89.1	29.9	31.4
Fe (mg/L)	0.54	1.94	0.29	0.27	0.65	1.22	0.44	0.28
Mg (mg/L)	4.93	29.2	8.23	9.21	9.02	35.4	15.5	15.1
Mn (mg/L)	0.05	0.11	0.07	0.15	0.28	0.58	0.71	0.26

Table 3. Manganese (mg/L) in surface and ground waters of Wayne County, WV (retreived from USGS-WRD QWDATA database and analyzed with STATIT) (Analysis by National Water Quality Laboratory, Arvada, CO)

a) Dissolved Mn in surface water (filtered through 0.45 um) (n=268)

Mean	0.161
Median	0.060
Minimum	<0.001
Maximum	2.000

b) Total Mn in surface water (n=187)

Mean	0.225
Median	0.130
Minimum	<0.010
Maximum	1.900

c) Dissolved Mn in ground water (filtered through 0.45 um) (n=86)

Mean	0.218
Median	0.100
Minimum	<0.001
Maximum	1.400

Table 4. Microorganisms settling on glass slides (slides left in water from 5/7/96-6/18/96 unless otherwise noted)

Sample number	Sample locality	Presence of oxidized Mn	pH (units)	Brown film with colorless rods	Brown holdfasts of L. discophora (singular or elaborate)	Brown rods (singular or colonial)	Brown filaments	Colorless rods	Colorless rod chains	Colorless cocci	Colorless filaments (sheaths flacid & filaments branched)	Colorless filaments (sheaths rigid & unbranched)	Red filaments	Red films	Red holdfasts	Cyanobacteria	Fungi	Diatoms (no. of species)	Other algae	Testate or atestate amoeba (no. of species)
Abbreviations					r=rare c=common a=abundant			s=short m=medium l=long										c=common a=abundant	unid.= unidentified	
QF-B	Rock shelter (discharge from mine)	present	2-3	--	--	--	--	s,m	--	cocci	filaments	long filaments	??	cocci	hold-fasts	--	--	--	unid. filamentous green	--
QF-D	NaOH pipe outlet	present	9	--	singular (c)	--	fila-ments	s,m,l	chains	cocci	filaments	long filaments	--	--	--	--	--	?	--	--
QF-F	Pond 2A	present	9	film	singular (r-c) elaborate	singular	--	s,m,l curving	chains	cocci	filaments	long filaments	--	--	--	rod chain; Spirulina	spores	4 (c-a)	?? (Mn coated)	--
QF-G	Black rocks at Pone 2A discharge	present	9-10	film	singular (a)	singular	--	s,m,l	--	cocci	filaments	long filaments	--	--	--	rod chain; Oscillatoria; Spirulina	hyphae	5 (a)	?? (Mn coated)	3
QF-H	Black rocks at Pond 3 discharge	present	9-10	--	singular (a) elaborate (r)	colonies	--	s,m,l curving	chains	cocci	--	short filaments	--	--	--	--	hyphae	9 (a)	unid. filamentous green (2 sp.); Coleochaete	2
Parker Branch-PB	Unaffected creek	present	6-8	--	singular (c) elaborate (r)	singular	fila-ments	s,m,l	--	cocci	filaments	long filaments	--	--	--	--	--	11 (a)	filamentous green (unid.)	6
Sandstone experiment-SS (5/1/96-8/21/96)	Mn and coal streaks in sandstone	present	--	--	singular (r)	--	--	s,m,l curving	chains	cocci	--	long filaments	--	rods	--	--	--	--	--	--

Table 5. Microorganisms and minerals in moist flocculates and crusts from Kiah Creek mining treatment operations

Sample number	Sample locality	Sample type	Dissolved Mn content (mg/L)	pH (units)	Distribution of Mn	Protozoans	Red tubes (bacteria-sized)	Brown cocci, rods, or filaments	L. discophora-type films (red or brown)	Colorless bacteria	Colorless filaments	Colorless colonizers of mineral grains	Cyanobacteria	Algae	Fungi	Other organic matter
Abbreviations						m=motile				m=motile n=non-motile	p=with PHB?			r=rare c=common a=abundant unid.=unidentified		
QFIN-2	Rock shelter (discharge from mine)	red precipitate on rocks	50-400	~2-3	large discrete particles	small colorless (m)	tubes	--	red	cocci (m); rods, cocci (n); magnetotactics?	--	rods, cocci	--	Ulothrix (r); unid. filamentous green; diatoms (r)	--	fecal pellets; pollen
QF2AD-3	Pond 2A	brown floc on plastic barrier	0.1-4.0	~9	small clumped particles in flocculate	small colorless (m); large striated colorless (m); testate	--	--	--	spirochaete (m); rods, cocci (n)	--	rods, cocci	globular & filamentous; Spirulina	Ulothrix; unid. filamentous green; diatoms (a); chrysophytes (filamentous & globular); cf. Draparnaldia	spores	pollen & spores
QF2ADT-4	Pond 2A	brown floc on leaves	20	~9	small & large discrete particles	medium colorless (m); planarian? (m)	--	cocci	brown	rods (m); rods, cocci (n)	filaments	rods, cocci	--	unid. filamentous green	hyphae, spores	leaf tissue; pollen; plant tissue
QF3D-5	Pond 3A discharge	red-brown layer on sandstone	0.1-4.0	~9-10	small & large discrete particles	radiolarian-type	--	--	red-brown	rods, cocci (m); rods, cocci (n)	--	rods, cocci	Oscillatoria	unid. filamentous green; diatoms (r)	hyphae	moss
QF3ADT-6	downstream from Pond 3A	loose brown floc	8-10	~9-10	none	--	--	filaments	red-brown	rods (m)	filaments (p)	rods, cocci	--	--	hyphae	plant tissue; pollen
QF3A-7	Pond 3A	loose white floc	0.1-15	~10	discrete patches	small & large colorless (m); large with projections (m)	--	rods	--	cocci (m & n)	--	cocci	--	Ulothrix? (r)	--	pollen

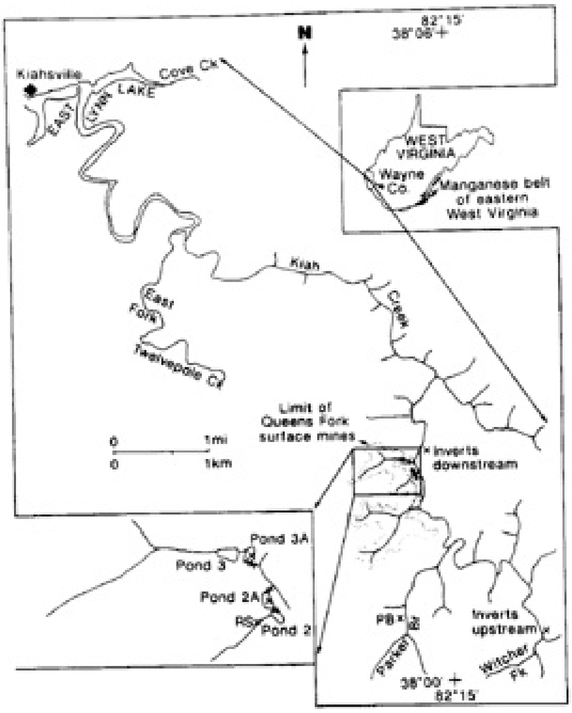

Figure 1. Locality map showing drainage, Queens Fork surface mines, and sample localities at Kiah Creek operations. (Distribution of Mn from King and Kirstein, 1989) (Abbreviations: Inverts = invertebrates and water chemistry, PB = Parker Branch, RS = Rock Shelter, X = collecting localities)

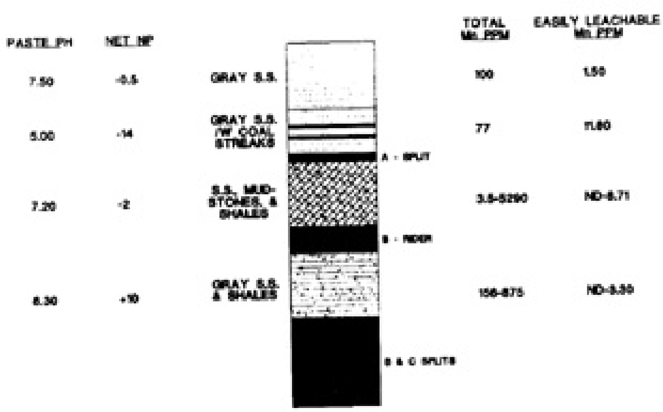

Figure 2. Typical lithologic column at Kiah Creek operations and analytical chemistry of selected units.

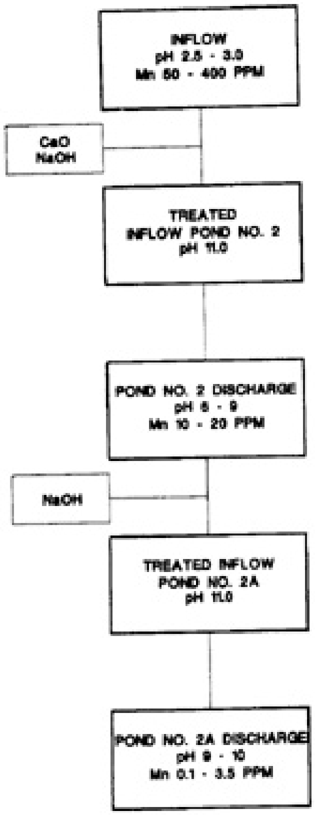

Figure 3. Chemical treatment at the Queens Fork operations, Kiah Creek.

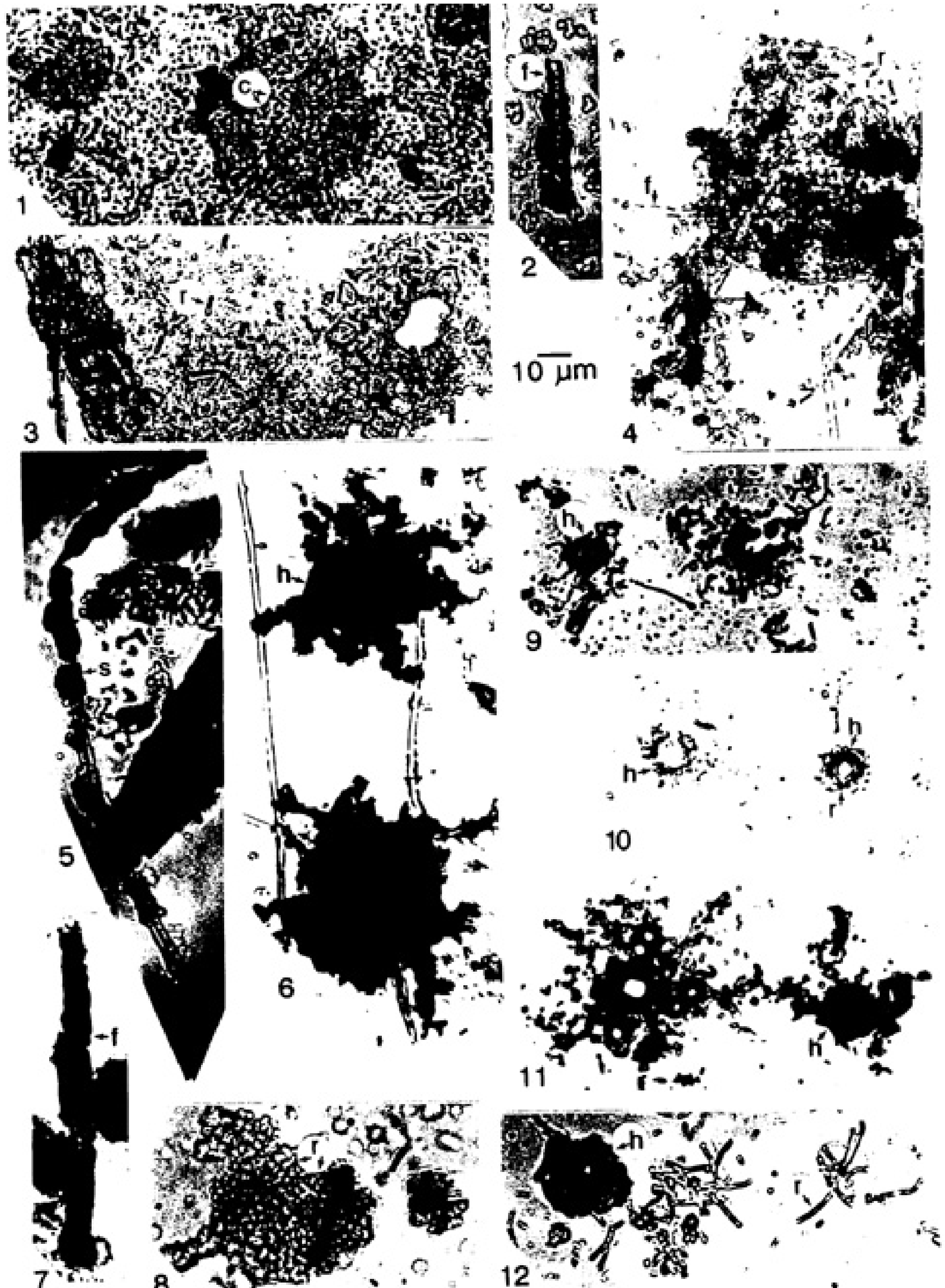

Figure 4. Bacteria on glass slides and in flocculates from the Queens Fork mine discharge and treatment ponds
Mine discharge--**1.** cocci (c) in red iron-oxide film (QF-B); **2.** filament (f) coated with red iron oxide from flocculate (QFIN-2)
NaOH treatment pipe--**3.** brown paste-like substance becoming colonized with rod-shaped (r) bacteria (QF-D)
Pond 2A--**4.** brown film with short rods (r) and colorless filaments (f) (QF-F); **5.** colorless filament colonized by clumps of brown cocci (cf. Siderocystis) (s) from flocculate (QF2DT-4); **6.** elaborate brown holdfasts (h) (QF-F)
Downstream from Pond 3A--**7.** brown filament (f) from flocculate (QF3ADT-6)
Pond 3A discharge--**8.** rod-shaped (r) bacteria in brown film from flocculate (QF3D-5); **9.** intact and dissolving brown Leptothrix discophora holdfasts (h) (QF-H); **10.** intact and dissolving brown L. discophora holdfasts (h); intact one covered with brown rod-shaped bacteria (QF-H); **11.** elaborate brown L. discophora? holdfasts (h); large one at right intact and large one at left collapsed (QF-H)
Sandstone experiment--**12.** intact brown holdfast (h) and long, curved rod-shaped (r) bacteria colonizing slide (SS)

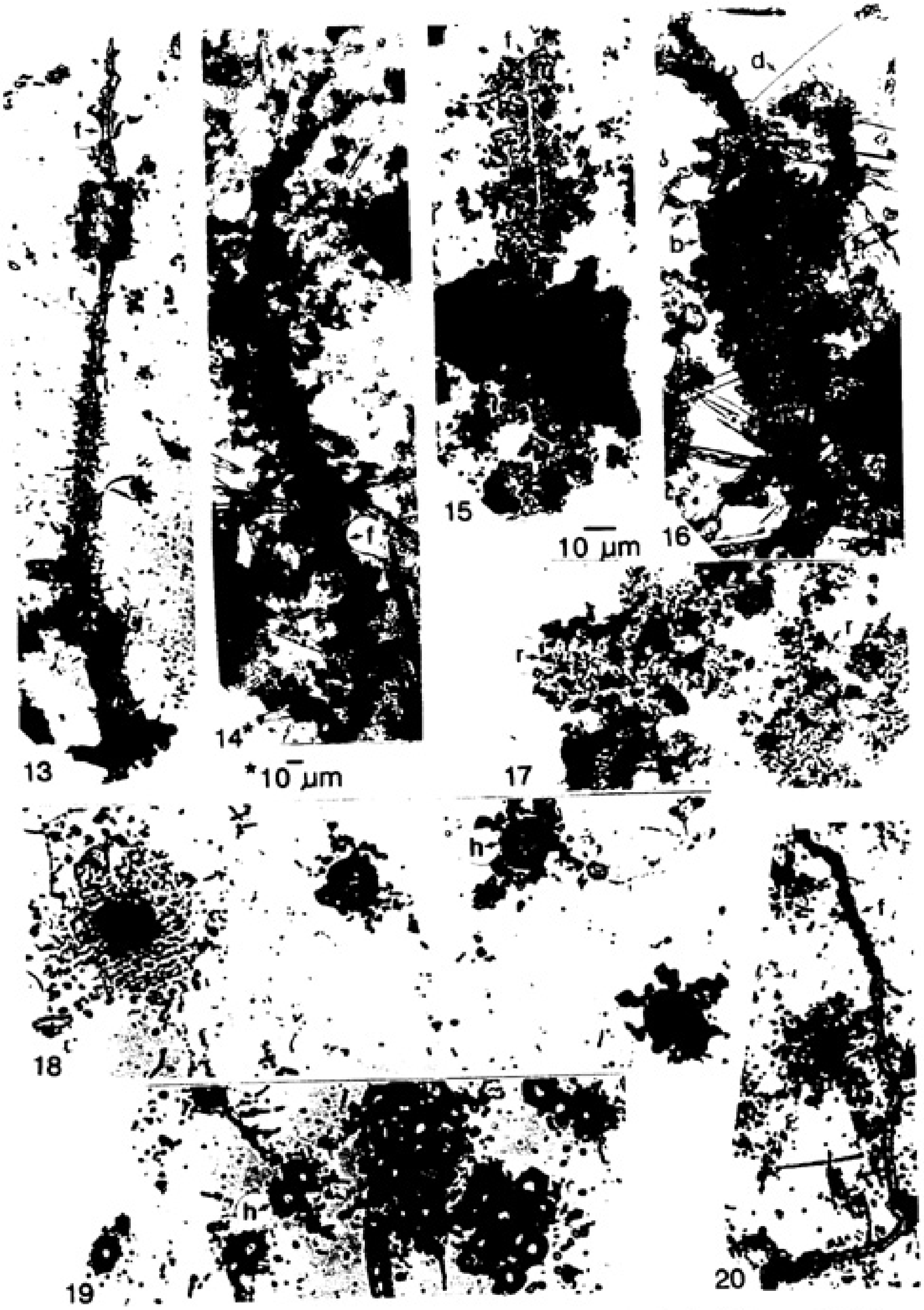

Figure 5. Bacteria on glass slides upstream and downstream from the Kiah Creek mining operations
Black rocks at Pond 2A discharge--**13.** colorless filament (f) being colonized by rod-shaped (r) bacteria and coated brown (QF-G); **14.** brown filament (f) coated with precipitate (QF-G); **15.** brown substance surrounding linear hole that was probably once a filament (f), suggesting the bacteria excreted an extracellular substance having Mn-oxidizing capability (QF-G); **16.** linear brown (b) coating too thick to determine its source; abundant diatoms (d) (QF-G); **17.** individual short rods (r) in brown film (QF-G)
Parker Branch control--**18.** sequence of Leptothrix discophora holdfasts (h) surrounded by short brown rods that are turning individual holdfasts into elaborate structures; the left holdfast surrounded by a film suggesting it was excreted extracellularly when bacterium was present (PB); **19.** group of collapsed L. discophora holdfasts (h) (PB); **20.** brown filament (f) having uniform thickness (PB)

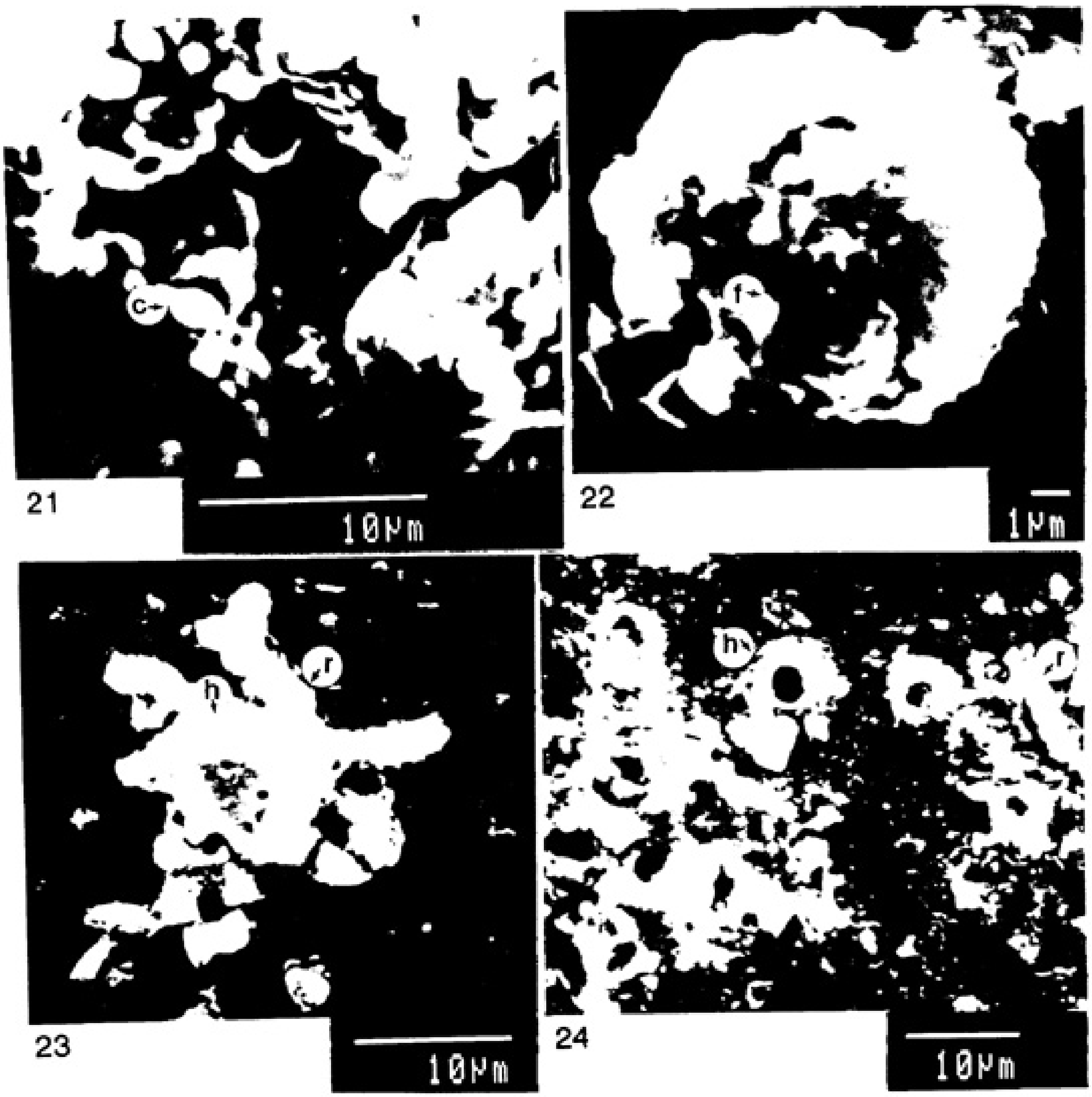

Figure 6. SEM images of microbial precipitates on glass slides
Mine discharge--**21.** iron-coated cocci (c) within red film (QF-B) (9,500x)
Pond 3A discharge--**22.** spherical intact Mn-coated holdfast of Leptothrix discophora having 1/2 μm-wide hole; note 1/2 μm-wide filament (f) adhering to holdfast (QF-H) (5,500x); **23.** L. discophora holdfast (h) surrounded by poorly defined Mn-coated rods (r) (rod-shaped bacteria?) (QF-H) (2,300x); **24.** group of collapsed L. discophora holdfasts (h) and rod-shaped (r) bacterium; note how holes appear to expand upon collapse of the spherical structures (QF-H) (2,000x)