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Ecogeochemistry of the subsurface food web at pH 0–2.5 in Iron Mountain, California, U.S.A.

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Abstract

Pyrite oxidation in the underground mining environment of Iron Mountain, California, has created the most acidic pH values ever reported in aquatic systems. Sulfate values as high as 120 000 mg l^{-1} and iron as high as 27 600 mg l^{-1} have been measured in the mine water, which also carries abundant other dissolved metals including Al, Zn, Cu, Cd, Mn, Sb and Pb. Extreme acidity and high metal concentrations apparently do not preclude the presence of an underground acidophilic food web, which has developed with bacterial biomass at the base and heliozoans as top predators. Slimes, oil-like films, flexible and inflexible stalactites, sediments, water and precipitates were found to have distinctive communities. A variety of filamentous and non-filamentous bacteria grew in slimes in water having pH values <1.0. Fungal hyphae colonize stalactites dripping pH 1.0 water; they may help to form these drip structures. Motile hypotrichous ciliates and bdelloid rotifers are particularly abundant in slimes having a pH of 1.5. Holdfasts of the iron bacterium *Leptothrix discophora* attach to biofilms covering pools of standing water having a pH of 2.5 in the mine. The mine is not a closed environment – people, forced air flow and massive flushing during high intensity rainfall provide intermittent contact between the surface and underground habitats, so the mine ecosystem probably is not a restricted one.

Introduction

Extreme environments can be anthropogenic as well as natural. At Iron Mountain in northern California, mining has enhanced the oxidation of pyrite to create subsurface waters having the most acidic pH values ever reported in nature (Alpers & Nordstrom, 1991). Acidic habitats are only now becoming objects of systematic ecological study around the world. Previous studies on acidic environments include Lackey (1938) and Joseph (1953) on coal mine drainage streams and soils in Indiana, Pennsylvania and West Virginia, and Ehrlich (1963) on acidic copper mine waters. Nordstrom & Southam (1997) published an extensive review of the geomicrobiology of sulfide mineral oxidation with more than 300 references.

The host rock of the Richmond Mine (Figure 1) near Redding, California, is the Balaklala Rhyolite, a felsic volcanic rock with little buffer capacity to offset acidity generated from fine grained sulfide minerals in the deposit (Alpers et al., 1994). During mining from the 1880s to the 1960s, approximately 12 million tons of ore were extracted yielding metallic Cu, Pb, Zn, Ag and Au, and sulfuric acid (Kinkel et al., 1956; Alpers et al., 1994; Nordstrom & Alpers, 1999a). Remediation efforts began in 1983 under the Superfund Program of the U.S. Environmental Protection Agency (Nordstrom & Alpers, 1990, 1999b).

Geochemical and mineralogical investigations have been the major thrust of previous field studies at Iron Mountain (Nordstrom, 1991; Alpers & Nordstrom, 1991; Alpers et al., 1994). These studies showed that drainage within the Richmond Mine had pH values ranging from 0 to 1.1, whereas the pH of drip waters may be as low as -3.6 (Nordstrom et al., 1991; Nordstrom & Alpers, 2000). Heat from mineral reactions keeps the water at 40 $\pm$5 °C in the tunnel. The silica content of this heated water was used to calculate the upper temperature limit that could exist in the mine. Nordstrom & Potter (1977) calculated 50 °C as the upper limit; additional data now suggest that temperatures could reach 65 °C, which puts the environment into the range of thermophilic bacteria.

Microbial genetic and fluorescence studies have revealed the presence of bacteria, Archaea and Eukarya in the mine. Rodgers (1996) and Rodgers et al. (1996) reported a total of 63 clones of 16S rRNA encoding genes from two samples. Diversity was very low. Many of the gene sequences retrieved from the mine water and pyrite sediment had >90% similarity to rRNA sequences of common soil bacteria (Edwards et al., 1999). *Acidimicrobium, Acidiphilium* and *Acidobacterium* were also present. Schrenk et al. (1998) identified *Leptothrix ferrooxidans* as the dominant iron- and sulfur oxidizer within the mine, and *Thiobacillus ferrooxidans* as the dominant iron-and sulfur-oxidizer at the mine discharge in samples from January 1997, but considerable variation occurred as the seasons changed (Edwards et al., 1999). Fluorescence in situ hybridization studies showed that *L. ferrooxidans* and *T. ferrooxidans* composed only a small proportion of the total microbial population (Edwards et al., 1999, 2000) and that Archaea dominated B-drift sediments in the summer along with abundant Eukarya.

The present study focuses on the ecology of microhabitats in the Richmond Mine. The objectives of the study were to characterize the full range of bacterial morphotypes and to identify any other viable organisms. Water samples were collected to help characterize the geochemical environment.

Materials and methods

Water samples were collected in acid-washed plastic ware and filtered on site using a disposable Nalgene hand-pumped filter assembly with a 0.2 μm pore size membrane using the methods in Wood (1976). Split samples for cations and trace metals were preserved by acidification with nitric acid. Major cations and trace metals were determined by inductively-coupled plasma optical emission spectroscopy (ICP-OES). Separate samples for anions were stored without acidification and analyzed by ion chromatography. The pH electrodes were conditioned in 0.1 N sulfuric acid for about 24 h prior to use in the field. A series of 10 sulfuric acid solutions were prepared over a range of concentrations for use as standard buffers. Where water was not sufficient for electrode immersion, pH estimates were made using indicator strips.

Microhabitats were chosen to provide samples of slimes, oil-like films, flexible and inflexible stalactites, sediments, water and precipitates. Identifications are based on descriptions in Pennak (1978) with help from S. Woelfl and G. Packroff (UFZ Centre for Environmental Research, Leipzig-Halle, pers. comm., 1998). Samples were collected in vials by sterile pipette or in jars using a variety of sterile scrapers.

Mine habitat

Mining and mineral processing have combined with climatic factors to intensify acid production at the Richmond Mine site. Annual rainfall averages 84 cm yr^{-1} and mean surface temperature averages 17 °C. The dry season lasts about 6 months (May–October), allowing build up of efflorescent salts within mine tunnels, drifts and stopes. The 6-month-long rainy season (November–April) begins with 2 months of intermittent rains, followed by intense rainfall events in the cool, winter months. Under this regime, the efflorescent salts are to a large extent flushed at the beginning of the rainy season, adding to the metal loading from this site. Ore extraction involved heavy blasting and drilling, which fractured the rock and opened conduits for air and water to infiltrate into easily weathered sulfide minerals. Under the seasonal climatic regime around the mine at about 800 m elevation, the surface vegetation is a fire adapted chapperal dominated by manzanita and live oaks. Willows line water courses; pines and spruce dominate the hillslope vegetation and shed pollen that enters the mine.

During the winter, large discharges often flow through the Richmond Mine tunnel (portal). Typical flow during the dry season is 75 l min^{-1} (Alpers et al., 1994); discharge reached a high of 8000 l min^{-1} following an intense rainfall in February 1998 (J.S. Cogliati, Stauffer Management Co., pers. comm., 1998). The mine workings are open to air circulation both at the bottom and at the top; the lower levels of the mine are drained by a horizontal tunnel and several of the collapsed stopes reach the surface, creating vents that discharge gases and heat. When people are conducting underground studies, air is introduced via a large fan, thereby providing more oxygen inflow.

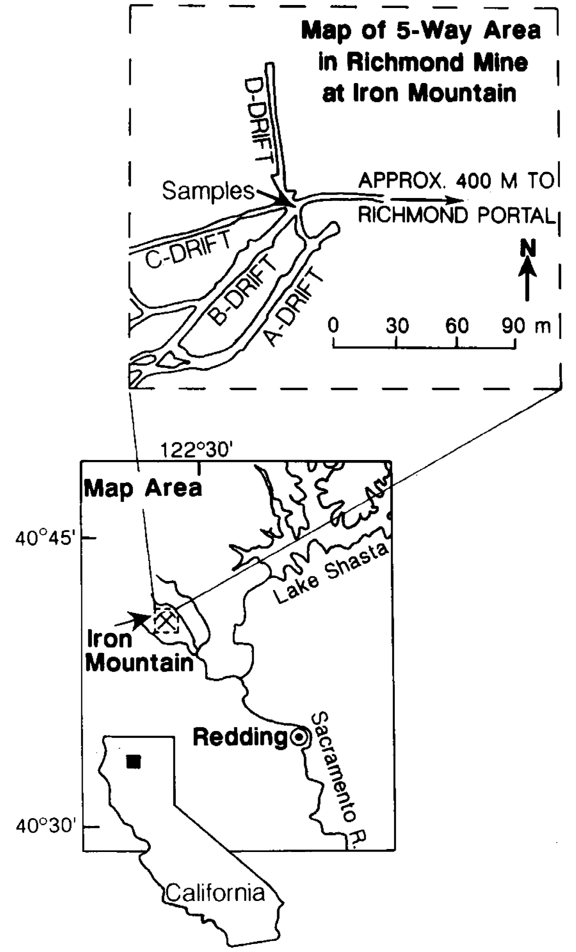

Figure 1. Location of study site and samples, Richmond Mine at Iron Mountain, California.

The field site lies 177 m below the mountain surface, within the 450 m-long and about 3 m high Richmond Mine tunnel. The tunnel leads to an open area called the 5-Way, where four drifts, named A, B, C and D, meet the main tunnel (Figure 1). In August,1996, when most of this research was performed, the tunnel floor was wet; pools of water stood at scattered localities. Weir dams held back pools at the B and C drifts. At the time of this study, stalactites of slime and yellow jarosite hung down from walls of the D drift, and stalactites and stalagmites of blue (cuprian) melanterite were forming in back areas of the tunnel with active ceiling drip.

Results

Sample locations, pH and types of bacteria, fungi, protozoans and microscopic animals are given in Table 1.

pH 0

Melanterite stalactites. Rod filaments (Figure 2-4) colonized a blue melanterite stalactite hanging from the D drift roof.

pH 0.5–0.8

White slime mat spreads out across water surfaces that have a visible current. The slime is as much as 1 mm thick. Inside the slime are cocci (Figure 2-3), elongate coccus filaments (filaments formed of spherical cocci) (Figure 2-1), and long (>5 μm) rod filaments (filaments formed of elongate rods). Video photography showed that a few singular cocci were motile. Most bacteria were fully enmeshed in the mat.

Green slime streamer filaments spread out and moved with the flowing water at a discharge of about 75 l/min. Streamers were made of coccus and rod filaments, many of which were entwined. Black poorly crystalline mineral phases (FeS_2) were distributed within the filaments (Figure 2-2).

pH 1

Pyritic sediments collect at the base of the C drift weir pool. The sediment contained filaments (Figure 2-5), many of which had adherent black (FeS_2) and brown (goethite?) poorly crystalline mineral phases of unknown composition (Figure 2-6). Cocci and rods were also noted and reported by Schrenck et al. (1998), who found *L. ferrooxidans* in abundances of 10^6 cells/mL.

Flexible stalactites, hanging from the D drift ceiling, contained a mass of fungal hyphae and thick-walled sporangia (Figure 3-10 and 11). Stalactites of undetermined pH had fungal spores (Figure 3-12) and hyphae (Figure 3-13).

pH 1.5

Pyrite gel was extracted from massive pyrite that was weathering in place on a wall at the D drift. A yellow jarositic layer coated the pyrite gel. Fungal hyphae and spores were present in the gel. Bdelloid rotifers were also observed moving through it.

Table 1. Richmond Mine bacteria, fungi and animals (–, not present; nd, not determined) (*10 Sep. 1996; †1 July 1998)

Sample no. & location	pH	Sample type	Bacteria motile	Bacteria non-motile	Fungi	Protozoa	Rotifers	Other
5* D drift	0	blue-green melanterite stalactite	cocci	rod & coccus filaments	–	–	–	–
2* C drift	0.63	green streamers	rods, cocci	rod filaments, bifurcating filaments	–	–	–	–
1* C drift	0.79	white slime	–	coccobacilli, vibrios, cocci, diplobacilli	–	–	–	–
3* C drift	0.79	white & pink slime	membrane-bound cocci	rod filaments	–	–	–	–
4* C drift	~1	pyrite sediment	rods	bifurcating rod filaments	–	–	–	–
7* D drift	~1	flexible yellow stalactite	cocci	bifurcating rod filaments	ascospores, brown sporangia, colorless hyphae	–	–	–
6* D drift	nd	jarosite stalactite	–	rod & cocci filaments, cocci, rods	spores, colorless hyphae	–	–	–
20† (107)	1.5	pyrite gel	cocci, rods	rods, rod filaments, filaments, vibrios, diplorods	spores, colorless hyphae	hypotrichous ciliates	bdelloids	–
10* D drift	nd	flexible slime stalactite	rods	rod filaments	brown sporangia, colorless hyphae	–	bdelloids	–
17† (CR1)	2	flexible slime stalactite	–	cocci	colorless hyphae, spores	hypotrichous ciliates	bdelloids	pollen
18† (105B)	2	thick jarosite stalactite	cocci	–	spores, hyphae (some bifurcating), brown sporangia	–	bdelloids	pollen, dead diatom?
21†D drift	~2	white/orange slime	cocci, rods	cocci & rods in film, *L. discophora*	spores, hyphae	hypotrichous and monad ciliates, rhizopod?	bdelloids	dead diatoms
19† (105A-5)	2	narrow jarosite stalactite	diplorods, spirochetes	cocci, rods, rod chains, rod and empty filaments	spores (2), colorless hyphae, brown sporangia	hypotrichous ciliates, heliozoan	bdelloids numerous	dead diatom, orange tube
8* D drift	2–2.5	red film on wall	rods, cocci	rods, rod filaments, and cocci in film, *L. discophora*	hyphae	monad ciliates, rhizopod	bdelloids	red tubes, dead diatom
11B* tunnel	~2.5	red film on pool	–	rods in film, *L. discophora* holdfasts	brown hyphae	–	–	dead diatom
12* tunnel	~2.5	yellow-white flocculate in pool	rods, vibrios	rare rod filaments	triplospores, brown sporangia	hypotrichous ciliates, heliozoan	bdelloids	–

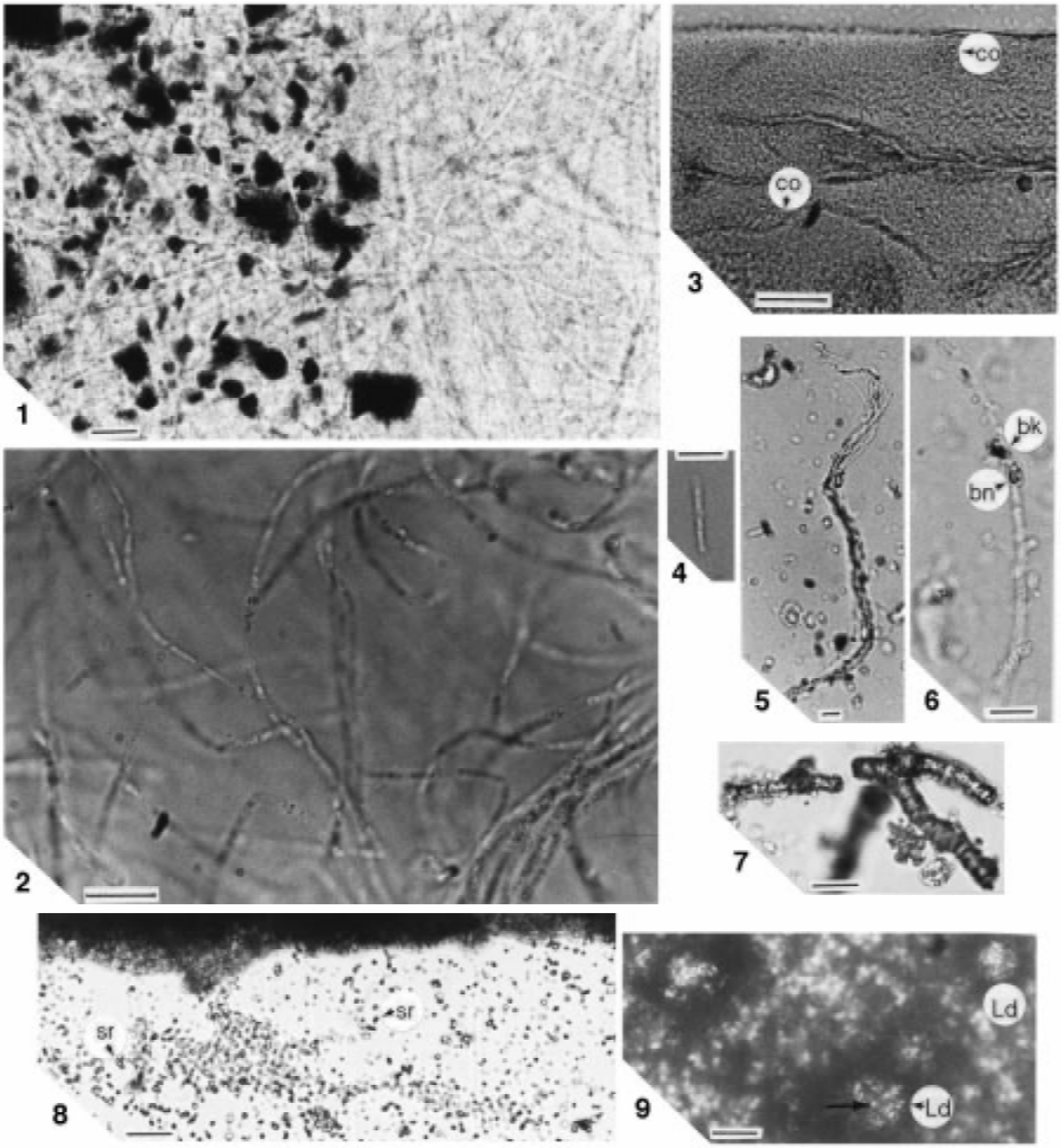

Figure 2. Photomicrographs of bacteria in the Richmond Mine, Iron Mt., California. (Scale bar 10 μm; sample identification number in parenthesis). 1. Mass of rod filaments with black (sulfide?) blebs (#3). 2. Mass of rod filaments (#2). 3. Cocci (co) within coherent membrane (#1). 4. Short rod filament (#5). 5. Entwined filaments (#4). 6. Rod filament with black (bk) (iron sulfide?) and brown (bn) (iron oxide?) blebs (#4). 7. Red tubes on iron oxide biofilm (#8). 8. Short rods (sr) on iron oxide biofilm (#11B). 9. *Leptothrix discophora* holdfasts (Ld) (#11B) (note opening where bacterium once attached-arrow).

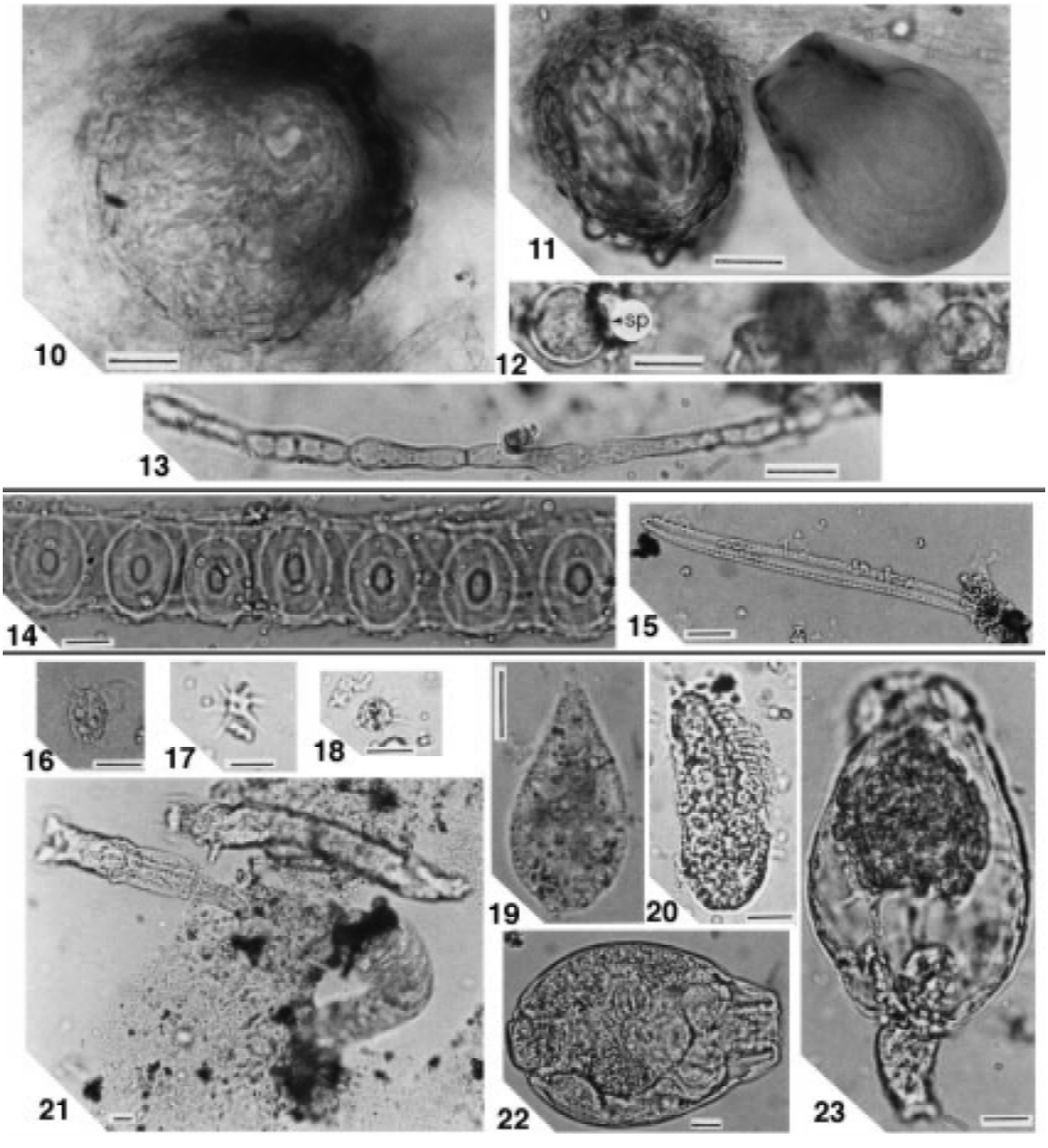

Figure 3. Photomicrographs of Richmond Mine fungi, exogenous carbon, and animals (Scale bar 10 μm; sample numbers in parentheses are keyed to Table 1). Fungi 10. Thick-walled sporangium (cf. ascomycete perithecium) (#7) (focus on ostiolar pore) 11. Thick-walled sporangium and hypha (#7) (left focus on striate morphology; right focus on neck of sporangium). 12. Spores (sp) (#10). 13. Hypha (#6). Exogenous carbon 14. Conifer wood cell (note bordered pits). 15. Diatom (*Gyrosigma*) (#21). Protozoans 16. Monad (cf. *Pithothorax*) (#21). 17. Rhizopod (cf. *Protomonas*) (#8). 18. Heliozoan (cf. *Sphaerophrya*) (#12). 19. Heterotrichous? ciliate (cf. *Blepharisma*) (#12). 20. Hypotrichous ciliate (cf. *Pleurotricha*) (#12). Rotifers 21. Three bdelloids (cf. *Rotaria*) (#8). 22. Bdelloid (#12). 23. Bdelloid (cf. *Mniobia*) (#21).

pH 2

Flexible jarosite stalactites and *hard jarosite-silica stalactites* hanging from the wall of the drifts had abundant fungal hyphae and spores. Furthermore, motile bdelloid rotifers and egg/cysts were present.

Whitish-orange slime on the D-drift wall had protozoan monads (Figure 3-16) and bdelloid rotifers (Figure 3-23).

pH 2-2.5

Hard red iron oxide film was scraped from the mine wall in the Richmond Mine tunnel. The film had red microbial-size tubes (Figure 2-7), hypotrichous ciliates, a rhizopod (Figure 3-17), and bdelloid rotifers (Figure 3-21).

Oil-like red film on pool of Richmond Mine tunnel contained numerous rods (Figure 2-8) and holdfasts of *L. discophora* (Figure 2-9). The presence of this taxon was confirmed by molecular analysis (Edwards et al., 1999). Fungal hyphae were also present.

Yellow flocculate under red oil-like film contained ciliates (Figure 3-19 and 20), a heterotrophic heliozoan (Figure 3-18), and bdelloid rotifers (Figure 3-22).

Carbon sources

Endogenous and exogenous sources of carbon are present in the mine. Endogenous sources are abundant rotting mine timbers, atmospheric CO_2 and remains of autotrophic and heterotrophic bacterial cells, sheaths and slimes. Exogenous sources are soluble humic substances dissolved in infiltrating soil water and particulate organic carbon such as diatoms (Figure 3-15) blown in by the fan and carried in on shoes. N and P have not been measured.

Geochemistry

Pyrite dominates the massive sulfide minerals (90–95%); lesser amounts of chalcopyrite along with galena, sphalerite, tennantite-tetrahedrite and pyrrhotite are present. Mining activities exposed large quantities of these minerals and created a hydrobiogeochemical ‘reactor’ that weathers about 4260 t pyrite every year and releases about 725 kg Cu, Zn and Cd every day. Fortunately, most (80–90%) of the metal loading to the streams is being remediated. The primary means of treatment is lime neutralization followed by high-density sludge separation.

Table 2. Chemical composition (mg l^{-1}) of Richmond Mine tunnel water

Sample No.	90WA103 Sep. (1990)[a]	Richmond Mine, Drift B at 5-way Sep. (1996)[b]	Samples at Richmond Mine portal discharge[a]
pH	0.48	0.4	0–1
water T °C	34.8	37.6	–
Total C_{org}		4.4	–
SO_4	118 000	105 000	20 000–120 000
Fe(total)	20 300	27 600	10 000–20 000
Al	2210	1980	1000–2200
Zn	2010	2460	1000–2400
Mg	821	1030	–
Cu	290	377	100–500
K	261	266	–
Na	251	233	–
Ca	183	248	–
SiO_2	165	127	–
As(total)	56.4	52.9	20–60
Cd	15.9	19.7	10–20
Mn	17.1	22.1	–
Ti	5.9	8.2	–
Sb	4.0	–	–
Pb	3.6	<5.1	2–5
V	2.9	2.2	–
≥1<2 B, Co, Sn			
<1 Ag, Ba, Be, Cr, Mo, Ni, Se, Sr, Tl			

[a] Alpers et al., 1992.
[b] 19 Sep. 1996.

Two water samples analyzed from the Richmond Mine (Table 2) were shown to be acid iron sulfate solutions with high concentrations of most metals. The typical range of concentration and pH for Richmond Mine portal effluent samples are similar to values within the mine.

Oxidized and reduced iron

The form of iron provides information about energy sources for autotrophs in the mine. The mine contains a very large reservoir of dissolved ferrous iron as well as solid pyrite; it also contains structures made of ferric iron. One pool in the tunnel was completely coated with a red oil-like biofilm that contained rods and distinct holdfasts of the iron bacterium, *L. discophora* (Figure 2-9). The microbial process that forms the biofilm and precipitates iron oxide is thought to be the result of metabolic reactions that do not involve energy transfer (Ghiorse & Ehrlich, 1992). Slime filaments floating on moving water had adherent black sulfide blebs suggesting that iron and sulfate reduction may occur in the mat. Slime filaments within the pyritic sediment had brown (goethite?) blebs, suggesting that iron oxidation was also an active process.

Discussion

The food web in the Richmond Mine shares aspects with communities reported from other highly acidic environments. However, this underground food web is based on microbial production rather than the primary photosynthesis that supports the ecosystems of highly acidified mining lakes such as in Germany (Klapper & Schultze, 1995; Nixdorf et al., 1998). Although predation was not observed, heliozoans probably form the top of the underground food web.

Bacteria were very diverse and were present in all microhabitats. Cocci, rods and filaments were collected in waters of all pH values and microhabitats. Fungi made their appearance at pH 1, holotrichous ciliates and bdelloid rotifers at pH $\geq$1.5, and rhizopods and heliozoans at pH $\geq$2–2.5. Rotifers have been observed before in pH 2.5 mine water (Green & Kramadibrata, 1988).

The slime mat bacteria are probably not restricted to the mine. The mat is flushed out during the wet season, but it reforms in April at the end of the rainy season and spreads out over ponded water surfaces in the dry season. The mat is also present at the discharge of the mine water, where sunlight is present, but no photosynthetic pigments were observed.

The presence of fungi in the slime stalactites is quite significant. It is possible that hyphae begin the process of stalactite formation by hanging down and focusing the dripping water. Eventually, the stalactite structure must harden as crystalline mineral phases are formed. Fungi, however, do not have known enzymes that function below pH 2 (W. Gross, written commun., 1998); therefore, the abundant sporangia of an unidentified ascomycete may be serving as resting phases during the dry season when pH values would be lowest. Perhaps the hyphae grow during the rainy season when infiltrating water may have higher pH values.

The holotrichous ciliates, rhizopods, bdelloid rotifers and heliozoans will probably prove to be cosmopolitan in their distribution. Although the mine is an underground habitat, it is open to surficial water, forced air and human activity.

The results from this study show a simple but unexpected underground food web that is based on iron-and sulfur-oxidizing bacteria and Archaea living at pH 0–2.5 in waters that contain numerous metals having concentrations as much as 120 000 mg l^{-1} sulfate and 27 600 mg l^{-1} iron. These organisms, coupled with additional exogenous carbon sources, support heterotrophic bacteria, as well as Eukarya. The unexpected presence of acidophilic or acid-tolerant fungi and motile protozoans, rotifers and heliozoans suggests that the biology, ecology and genetics of other underground mining environments can be expected to yield useful new insights into ecogeochemical interactions where bacterial biomass forms the base of the food web.

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