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EXTREME ACIDOPHILES:
Freshwater Algae Associated with Acid Mine Drainage

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1. Introduction

1.1. DESCRIPTION OF THE HABITAT

Acid mine drainage (AMD) is a phenomenon commonly associated with mining activities throughout the world. This acidification is the consequence of sulfides in rock strata becoming exposed to water and oxygen (see Section 1.2). The low pH of AMD-contaminated water bodies may resemble that of some naturally occurring freshwater systems that harbor acidophiles. For example, regions of the West Coast of the South Island of New Zealand have streams and rivers with naturally low pH produced by leaching of fumic and fluvic acids from podocarp rainforests, as well as artificially low pH systems caused by AMD (Collier et al., 1990). Ancient low-pH environments generated by volcanism may have been crucial for the origin of life on Earth (e.g., Holm and Andersson, 2005; Phoenix et al., 2006), and thus habitats resembling AMD have probably existed for billions of years. Distinctly, however, extremely acidic habitats from anthropogenic sources are associated with a massive burden of spoil and heavy metals. AMD began during the industrial revolution, and now accounts for most of the extremely acidic habitats worldwide (Johnson, 1998).

AMD can occur in both surface runoff (particularly in opencast mines) and in the groundwater associated with underground mines. Both surface runoff and groundwater frequently supply and contaminate streams (Harding and Boothroyd, 2004), and lakes within the catchment (Wicks et al., 1991; Niinioja et al., 2003; Tittel et al., 2005). Coastal mining may have significant local impacts on marine intertidal and coastal ecosystems. However, Johnson (1998) defined acidophiles as organisms with growth optima at or below pH 3.0, which would probably exclude much of the marine environment except at highly impacted river mouths and close intertidal zones. The impact of AMD in marine ecosystems has been the subject of much research that is beyond the scope of this chapter. Here, we focus on algal communities in freshwater systems impacted by AMD.

1.2. CHEMISTRY AND BIOCHEMISTRY OF AMD

Freshwater environments exposed to AMD typically have extremely low pH and high concentrations of dissolved metals (Table 1). In the following discussion we will refer to pH, which although not exactly the same as "acidity," is frequently synomized (Johnson, 1998; Harding and Boothroyd, 2004). In freshwater ecosystems, AMD contamination frequently leads to the collapse of benthic communities through the elimination of sensitive taxa. A useful review of the environmental impacts of AMD, including an overview of chemical, physical, biological, and ecological impacts and details of the effects of metal toxicity, sedimentation, and breakdown of buffering systems in the context of community structure, is given in Gray (1997).

The processes generating acidity in AMD have been the subject of some debate (Evangelou, 1995; Sand et al., 1995; Johnson, 1998). However, consensus has emerged that the ferric ion acts as the major oxidant of the mineral according to the following equation:

$$FeS_2 + 6Fe(H_2O)_6 + 3H_2O \rightarrow Fe^{2+} + S_2O_3^{2-} + 6Fe(H_2O)_6^{2+} + 6H^+.$$
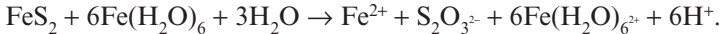
The reactions described by Singer and Strumm (1970) show that pyrites can remain in their reduced state in undisturbed strata as long as they are not exposed to oxygen (Gray, 1997). Currently, mining is the most common means by which this exposure occurs. However, bacteria may also play a role, through subsequent

Table 1. A selection of reported values for pH and dissolved metals (mg L^{-1}) from AMD-impacted streams, and Maximum Contaminant Levels (MCLs) for drinking water according to the U.S. Environmental Protection Agency.

Country	pH	Fe	As	Cu	Zn	Al	Mn	Reference
USA	0.83	50[a]	8.5	4.1	14	–	–	Baker et al. (2004)
Japan	2.3	276	1.67	–	–	69	–	Sasaki et al. (2005)
England	3.4	15.0	–	–	–	–	7.8	Singh et al. (1999)
England	–	140	2.5	0.4	75	50	23	Barley et al. (2005)
Germany	2.1–	390–	–	0.004–	1.69–	22–	1.3–	Lessmann et al. (1999)
(Lake)	2.5	670		0.047	2.91	60	43.5	
New Zealand	2.7–	0.2–	–	–	–	0.01–	–	Winterbourn et al.
	6.2	32.6				35.5		(2000)
New Zealand	2.8	36.5–	–	0.012–	0.53–	–	0.43–	Novis (2005)
		37.0		0.016	0.54		0.44	
New Zealand	2.7–	0.1–	0.18–	–	0.92–	159–	–	Harding (2006)
	7.7	84.5	0.001		0.001	0.04		
South Africa	1.88	98.95	–	3.49	7.16	–	–	Van Hille et al. (1999)
U.S. Recommended level	6.5– 6.8	<0.3	<0.01	<1.3	<5	<0.05	<0.05	USEPA (2006)

[a]Fe^{3+} only.

biological oxidation of pyrites (Leduc and Ferroni, 1994; Marchand and Silverstein, 2002).

The buffering capacity of the receiving water, and the extent to which the water body can dilute the contamination, largely influence the impact of AMD (Gray, 1997). This is partially because the solubility of metal ions varies greatly with pH (Harding and Boothroyd, 2004) and also because dilution may reduce metal concentrations while not markedly influencing pH. At higher pH (>4.0), precipitation of metal hydroxides can smother biota with precipitates, whereas at lower pH the toxicity of dissolved metals, which can cross membranes (Van Ho et al., 2002), combined with acidity, may have the greatest impact. This complexity of impact has led to a view that AMD is "multifarious," affecting organisms in "numerous interactive ways" (Gray, 1997).

The impact of strong acidity on biota is associated with the conversion of carbonate and bicarbonate to carbonic acid (Harding and Boothroyd, 2004). This has two effects. First, a low-pH system may be more sensitive to further addition of AMD, since buffering capacity to moderate the pH is lost. Second, the absence of bicarbonate deprives autotrophs of inorganic carbon (although dissolved CO_2 may be more rapidly replenished from the atmosphere at low pH; Gross, 2000).

Metals can be divided into those that are required by organisms in small quantities, such as copper and zinc which are essential in some biochemical reactions (e.g., Raven et al., 1999), and those of no known biological utility, such as mercury and cadmium (Pinto et al., 2003). The toxicity of heavy metals to algae and other organisms appears to be due to the generation of reactive oxygen species (ROS; Pinto et al., 2003). An example of this toxicity is the generation of peroxides, which can disrupt the plasma membrane by lipid peroxidation (Macfarlane and Burchett, 2001).

Most strongly acidic environments are also oligotrophic, being nutrient deficient (Johnson, 1998). Because sulfate is a product of the oxidation of metal sulfides (Singer and Strumm, 1970), it is invariably high in AMD-contaminated waters; however, other inorganic nutrients used by plants and algae may be very low, particularly inorganic nitrogen (<0.04 mg NH_4–N L^{-1}, Novis, 2005). Given that carbon limitation may also occur, nutrient limitation may be a significant additional problem for algae in AMD-contaminated waters.

The resistance of many algae to AMD is thought to be due to their ability to complex metals outside the cells, preventing entry to the cytoplasm. Laboratory studies showed an increase in extracellular polymeric substance (EPS) produced by *Chlorococcum* sp. and *Phormidium* sp. as a response to increased copper and zinc (Garcia-Meza et al., 2005). In seawater, *Nitzschia closterium* was also found to complex copper outside the cell membrane (Stauber et al., 2000). However, this mechanism is not universal: a study of *Oocystis nephrocytioides* indicated that resistance to copper was due to accumulation and sequestration in thylakoids, with adsorption to the cell surface being less important (Soldo et al., 2005).

2. Diversity of Algae Associated with AMD

Acidophilic bacteria (chemolitho-autotrophs) have been the focus of much attention in AMD-contaminated waters (see review by Johnson, 1998). However, a large number of algal taxa have been documented in AMD exposed to light (Table 2 and references therein). In severe AMD with stable flow conditions

Table 2. Freshwater algae associated with AMD.

Taxon[a]	pH range in study	Habitat (country)	Reference
Cyanobacteria			
Phormidium sp.	8.0	Lake (Mexico)	Garcia-Meza et al. (2005)[b]
Hapalosiphon hibernicus W. & G.S. West	2.5–7.7	Stream (Sarawak)	Douglas et al. (1998)[c]
Euglenids			
Euglena mutabilis Schmitz	≥1.41	Streams (multiple countries)	Whitton and Diaz (1981)[d]
	1.5–2.4	Stream (Spain)	Sabater et al. (2003)
	2.0–5.0	Streams (USA)	Brake et al. (2001a, b)
	2.5	Stream (Sarawak)	Douglas et al. (1998)[c]
	2.6–3.1	Lake (Germany)	Kapfer (1998)
	2.5–4.7	Stream (France)	Casiot et al. (2004)
	2.1–2.5	Lake (Germany)	Lessmann et al. (1999)
	2.3–2.9	Lake (Germany)	Lessmann et al. (2000)
Euglena sp.	2.5–7.3	Stream (Sarawak)	Douglas et al. (1998)[c]
Phacus curvicauda Svirenko	2.5–5.8	Stream (Sarawak)	Douglas et al. (1998)[c]
Lepocinclis sp.	2.3–2.9	Lake (Germany)	Lessmann et al. (2000)
Chromists: chrysophytes			
Chromulina sp.	2.3–2.9	Lake (Germany)	Lessmann et al. (2000)
Ochromonas sp.	2.6	Lake (Germany)	Kamjunke et al. (2004)
	2.3–2.9	Lake (Germany)	Lessmann et al. (2000)
	2.6–3.6	Lake (Germany)	Wollmann et al. (2000)
Chromists: cryptomonads			
Cryptomonas ovata Ehrenb.	2.5–5.7	Stream (Sarawak)	Douglas et al. (1998)[c]
Cyathomonas sp.	2.3–2.9	Lake (Germany)	Lessmann et al. (2000)
Rhodomonas minuta Skuja	2.3–2.9	Lake (Germany)	Lessmann et al. (2000)
Chromists: diatoms			
Eunotia bilunaris (Ehrenb.) Mills	2.5–7.7	Stream (Sarawak)	Douglas et al. (1998)[c]
Eunotia exigua (Bréb. Ex Kütz.) Rabenh.	2.3–2.9	Lake (Germany)	Lessmann et al. (2000)
Eunotia hexaglyphis Ehrenb.	2.5	Stream (Sarawak)	Douglas et al. (1998)[c]
Eunotia incisa Greg.	2.5–7.7	Stream (Sarawak)	Douglas et al. (1998)[c]
Eunotia pectinalis (Dillw.) Rabenh.	2.5–5.7	Stream (Sarawak)	Douglas et al. (1998)[c]

(*Continued*)

Table 2. Freshwater algae associated with AMD—cont'd.

Taxon[a]	pH range in study	Habitat (country)	Reference
Eunotia sudetica O. Müll.	2.5	Stream (Sarawak)	Douglas et al. (1998)[c]
Frustulia rhomboides (Ehrenb.) De Toni	2.5–7.6	Stream (Sarawak)	Douglas et al. (1998)[c]
F. rhomboides var. *crassinervia* Rabenh.	2.5–7.6	Stream (Sarawak)	Douglas et al. (1998)[c]
Navicula mutica Kütz.	2.5–7.3	Stream (Sarawak)	Douglas et al. (1998)[c]
Nitzschia acicularioides Hust.	2.5	Stream (Sarawak)	Douglas et al. (1998)[c]
Nitzschia sp.	2.3–2.9	Lake (Germany)	Lessmann et al. (2000)
Nitzschia tubicola	Not available	Stream (USA)	Brake et al. (2004)
Pinnularia acoricola Hust.	1.5–2.4	Stream (Spain)	Sabater et al. (2003)
	2.3–2.9	Lake (Germany)	Lessmann et al. (1999)
Pinnularia braunii (Grunow) Cleve	2.5–7.6	Stream (Sarawak)	Douglas et al., (1998)[c]
Surirella tenuissima Hust.	2.5	Stream (Sarawak)	Douglas et al. (1998)[c]
Synedra sp.	2.5	Stream (Sarawak)	Douglas et al. (1998)[c]
Chromists: dinoflagellates			
Gymnodinium sp.	2.6–3.6	Lake (Germany)	Wollmann et al. (2000)
Chromists: xanthophytes			
Tribonema sp.	3.1–3.4	Stream (New Zealand)	Winterbourn et al. (2000)[e]
Plantae: chlorophytes			
Chlamydomonas acidophila Negoro	2.5–3.3	Lake (Germany)	Tittel et al. (2005)
Chlamydomonas botryopara Rodhe & Skuja	2.5	Lake (Germany)	Woelfl et al. (2000)
Chlamydomonas sp.	2.6	Lake (Germany)	Kamjunke et al. (2004)
	2.3–2.9	Lake (Germany)	Lessmann et al. (2000)
	2.6–3.6	Lake (Germany)	Wollmann et al. (2000)
Chlorococcum sp.	8.0	Lake (Mexico)	Garcia-Meza et al. (2005)[b]
Microspora tumidula Hazen	2.6–3.3	Stream (USA)	Verb and Vis (2001)[f]
Microspora sp.	3.1–4.2	Stream (New Zealand)	Winterbourn et al. (2000)[e]
Nanochlorum sp.	2.3–2.9	Lake (Germany)	Lessmann et al. (2000)
Oedogonium sp.	2.5–7.9	Stream (Sarawak)	Douglas et al. (1998)[c]
Pediastrum tetras (Ehrenb.) Ralfs	2.5–5.8	Stream (Sarawak)	Douglas et al. (1998)[c]
Scourfieldia cordiformis Takeda	2.3–2.9	Lake (Germany)	Lessmann et al. (2000)
Stigeoclonium sp.	8.2	Stream (Poland)	Pawlik-Skowrońska (2001)
Stigeoclonium tenue Kütz.	8.2	Stream (Poland)	Pawlik-Skowrońska (2003)[b]
Trentepohlia annulata Brand	2.5	Stream (Sarawak)	Douglas et al. (1998)[c]
Ulothrix sp.	3.0–11.0	Stream (USA)	Rousch and Sommerfeld (1999)

(*Continued*)

Table 2. Freshwater algae associated with AMD—cont'd.

Taxon[a]	pH range in study	Habitat (country)	Reference
	3.7–5.4	Stream (USA)	Niyogi et al. (1999)
	n.d.	Stream (USA)	Tate et al. (1995)
	2.7–4.1	Stream (New Zealand)	Winterbourn et al. (2000)[5]
Plantae: charophyceans			
Actinotaenium cucurbitinum (Biss.) Teiling	2.5–5.7	Stream (Sarawak)	Douglas et al. (1998)[c]
Klebsormidium acidophilum Novis	2.8	Stream (New Zealand)	Novis (2006)
Klebsormidium flaccidum (Kütz.) P.C. Silva, Mattox & W.H. Blackw.	1.5–2.4	Stream (Spain)	Sabater et al. (2003)
Klebsormidium rivulare (Kütz.) M.O. Morison & Sheath	2.9–5.6	Stream (USA)	Stevens et al. (2001)
	≥2.30	Streams (multiple countries)	Whitton and Diaz (1981)[d]
	1.5–2.4	Stream (Spain)	Sabater et al. (2003)
	2.6–3.3	Stream (USA)	Verb and Vis (2001)[f]
Klebsormidium sp.	2.2–3.4	Stream (Australia)	Lottermoser et al. (1999)
Mougeotia sp.	1.5–2.4	Stream (Spain)	Sabater et al. (2003)
	2.5–7.9	Stream (Sarawak)	Douglas et al. (1998)[c]
Spirogyra sp.	2.5–7.9	Stream (Sarawak)	Douglas et al. (1998)[c]
Plantae: rhodophytes			
Galdieria sulphuraria (Galdieri) Merola	1.5–1.9	Stream (Spain)	Gross and Gross (2001)
Unidentified red alga	0.76–1.38	Stream (USA)	Baker et al. (2004)

[a]Major groups follow Keeling (2004); traditional names are used for subgroups

[b]Chemical analysis of the mine water in these studies indicates that bicarbonate was responsible for the high pH

[c]Although Douglas et al. (1998) considered all their sites to be affected by (or at least associated with) mining activities, the range of pH of these sites was 2.5–8.7. Taxa included here were present in sites with pH < 3.0 (in fact no sites had a pH between 2.6 and 4.9, indicating that their sites J02 and J8 were much more heavily impacted than the others)

[d]Whitton and Diaz (1981) list many taxa from a range of acidic environments. Unfortunately, for most of these it is not clear which come from AMD-affected sites, and which from geothermal acid streams

[e]Only taxa from Winterbourn et al. (2000) that occurred in streams of pH < 4.0 and Fe concentration of > 1.0 mg L^{-1} are included

[f]Verb and Vis (2001) noted 40 taxa, but regarded only the 2 included here as indicative of AMD contamination

acidophilic algae can dominate the streambed. These organisms do not appear to have received a synthesis previously. A selection of taxa is shown in Fig. 1.

Many groups of algae have been reported from AMD (Table 2), but some taxa are much more common than others, for example, *Euglena* and *Klebsormidium* spp. This suggests cosmopolitanism. Cyanobacteria, which commonly dominate natu-

Figure 1. A selection of filamentous algae collected from streams draining abandoned mine adits in Westland, New Zealand. From top to bottom *Klebsormidium acidophilum*, *Microspora* sp., *Mougeotia* sp., and *Tribonema* sp. Scale bar = 10 μm.

rally acidic thermal springs, are very poorly represented in AMD. Likewise, thermo-acidophilic red algae (the unicellular Cyanidiales) are well documented from natural acidic springs, yet rhodophytes are almost unknown from AMD-contaminated habitats (Barbier et al., 2005). One strain of *Galdieria sulphuraria* is known from mining environments (Gross and Gross, 2001). Environmental PCR of samples from AMD sites with water temperatures of 30–50°C revealed two clones with 75% sequence identity to the cyanidialean *Cyanidioschyzon merolae* (Baker et al., 2004). Therefore, it seems likely that a greater diversity of red algae may be present in mining environments than has been identified so far.

Together, members of the "green lineage" (as defined by Nozaki et al., 2003) are the most diverse and widely reported taxa growing in AMD. Two genera in particular, *Euglena* and *Klebsormidium*, seem almost ubiquitous and are frequently dominant where they occur. Other groups appear to be locally common, such as chrysophytes, all the records of which come from mining lakes in Germany. Undoubtedly, this is partly due to a lack of systematic surveys in many habitats, although the paucity of records of protists in AMD-contaminated habitats has perhaps been overstated by some authors (e.g., Brake et al., 2001b; Baker et al., 2004).

A number of diatom species are recorded from AMD-contaminated freshwaters. Given the advantages of easy preservation and identification to species level from vegetative material, some authors have advocated their potential as indicators of AMD contamination (Nakanishi et al., 2004). They also give a rare example of periodicity in the simplified AMD community: the dominance of *Nitzschia tubicola* under certain conditions leads to the development of stromatolites, marking layers of alternately dominant diatom and euglenoid biomass (Brake et al., 2004).

3. Taxonomic Issues and Evolution of AMD-Tolerant Taxa

The presence of algae in these extreme environments raises the issue of their evolution and dependence on these environments. Is growth by algae in AMD-contaminated habitats facultative or obligate? Floristic surveys rarely provide us with sufficient information to address this issue, but some evidence is available from more detailed studies on small groups of organisms. *Euglena mutabilis*, for example, is widely reported from AMD-contaminated habitats at low pH (Table 2). The growth of this species is optimal between pH 3.0 and 3.5, and it tolerates high sediment loading (Brake et al., 2001a, b), and yet organisms also ascribed to *E. mutabilis* are reported from environments of much higher pH. Comparison of *E. mutabilis* and *E. gracilis* has shown that both species are acid tolerant – in fact the latter outcompeted the former at pH values typical of AMD – but *E. mutabilis* was far more tolerant of several heavy metals including aluminum, nickel, iron, and cadmium, although not copper (Olaveson and Nalewajko, 2000). Therefore, the success of *E. mutabilis* in AMD systems may be due to recent acquisition of heavy metal tolerance rather than a species-specific ability to cope with low pH.

   Perhaps the best documented example of preferential growth in AMD is that of *Klebsormidium rivulare*, which also illustrates the complexity of nomenclatural issues. The type specimen was collected from freshwater, apparently without influence of AMD, and named *Hormidium rivulare* (Kützing, 1845). However, Lokhorst (1996) identified this specimen as *Ulothrix implexa* (Kütz.) Kütz., suggesting that later transfer of *H. rivulare* to *K. rivulare* was inappropriate. The description provided by Kützing (1845) is also insufficient to distinguish *Klebsormidium* from several other genera, including *Ulothrix* and *Uronema*. In fact, ultrastructural study, requiring modern technology, is desirable in order to do this (e.g., Marchant et al., 1973; Lokhorst and Star, 1980, 1985; Lokhorst, 1996; Novis, 2006). The first "modern" study of *K. rivulare*, using ultrastructural data, was that of Morison and Sheath (1985). Their electron micrographs show that their specimens conform to the genus *Klebsormidium*, and hence differ from the *H. rivulare* of Kützing (1845). A number of subsequent studies (Table 2) from AMD-contaminated sites applied the name *K. rivulare* to similar taxa, perhaps because the material of Morison and Sheath (1985) was collected from an acidic stream (pH 4.3–5.8, Steinman and Sheath, 1984).

   However, it is now clear that *Klebsormidium* from AMD-contaminated sites differs from the *K. rivulare* of Morison and Sheath (1985), at least in some cases. Comparative culturing of a species from Sullivan West Mine, New Zealand, with *Klebsormidium dissectum* isolated from higher-pH environments showed that the pH optimum for the acidophile was 2.4–3.4, whereas the optimum for *K. dissectum* was 4.8–6.2 (Fig. 2). This clearly differs from the situation in different species of *Euglena*. Furthermore, the acidophile differed consistently in diameter from *K. rivulare*, and lacked typical field morphology over the pH range specified by Steinman and Sheath (1984). The new species *Klebsormidium acidophilum* was justified by
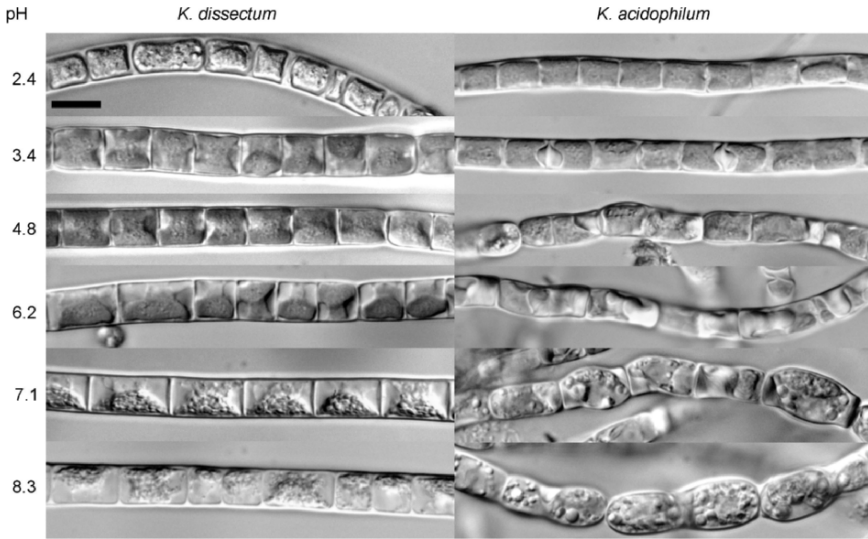
Figure 2. Morphology of two species of *Klebsormidium* grown for 3 weeks at different pH (left). *K. dissectum* was isolated from a stream of pH > 7.0; *K. acidophilum* from the stream from Sullivan West Mine, pH 2.8. Scale bar = 10 µm. Copyright (2006) International Phycological Society. From *Phycologia*, by P.M. Novis (2006). Reprinted by permission of Alliance Communications Group, a division of Allen Press, Inc.

these observations. *Rbc*L gene sequences indicated that genetic variation of *K. acidophilum* was nested within the variation of *K. dissectum* from local pH-neutral habitats (Novis, 2006). Therefore, AMD-tolerant taxa may evolve from local, less extreme habitats, contrasting with a view that organisms that successfully colonize AMD, such as *K. rivulare* and *E. mutabilis*, are cosmopolitan taxa. Testing this idea, using molecular comparison of AMD and pH-neutral taxa from local areas in different parts of the globe, constitutes important future research.

4. Structure of Algal Communities in AMD-Impacted Ecosystems

For algae that are able to colonize these extreme environments, AMD-contaminated waterways may offer some unique advantages. In particular, streams that discharge from mine adits are often unaffected by the disturbance of high rainfall and flooding (e.g., Trumm et al., 2005), which might otherwise limit growth. Furthermore, the paucity of grazing benthic invertebrates and usually the total absence of fish, crayfish, and other possible benthic grazers may allow the development of very high biomass, despite slow growth rates due to low nutrients and toxic contaminants. This is particularly important in systems where growth rates must exceed the rate of iron precipitation onto biomass for sustained colonization of the site to be viable (Harding and Boothroyd, 2004).

## 4.1. INTERACTIONS BETWEEN PROTISTS AND BACTERIA

Studies of acidophilic flagellates (*Eutreptia/Bodo* spp.), a ciliate (*Cinetochilium* sp.), and an amoeba (*Vahlkampfia* sp.) from AMD confirmed that these grazed on acidophilic bacteria (Johnson and Rang, 1993). Although photosynthetic euglenoids are mixotrophic and also capable of grazing on bacteria, and depend on associated microbial floras for vitamins B_1 and B_{12} (Graham and Wilcox, 2000), ecological relationships between the commonly reported AMD organism *E. mutabilis* and bacterial communities in these habitats are little studied. The AMD tolerance of *E. mutabilis* from Green Valley, Indiana, USA, falls within habitat preferences of sulfur- and iron-oxidizing bacteria published elsewhere (McIntosh et al., 1997; Brake et al., 2001b). Given the prolific growth of these bacteria at some sites (e.g., Johnson, 1998) and the carbon limitation for autotrophs at low pH (Tittel et al., 2005), this finding may partially explain the high frequency of colonization of AMD habitats by euglenoids.

Some bacteria are strongly implicated in the biological oxidation of pyrites, and hence their activities influence the function of AMD ecosystems. Surprisingly, interactions between bacteria and photosynthetic protists (i.e., eukaryotic algae) that are obligately autrophic are poorly documented in AMD. Stable consortia have been formed between algae and bacteria in the treatment of industrial wastewater containing heavy metals and hydrocarbons, although the pH tolerance of these organisms was not reported (Safonova et al., 2006). Algal biomass may facilitate bacterial sulfate reduction in mine discharge in artificial wetlands (Russell et al., 2003; see Section 5.2).

4.2. INTERACTIONS BETWEEN ALGAE AND INVERTEBRATES

The diversity of benthic invertebrate taxa is frequently greatly reduced in AMD-impacted systems, and can be correlated with stream pH (Fig. 3). A number of benthic freshwater invertebrates feed either partially or wholly on epilithic algae. Within New Zealand, these grazing invertebrates are usually dominated by Mollusca (snails) and insects, for example, Ephemeroptera (mayflies), Trichoptera (caddisflies), and Chironomidae (midges). In North America, several fish species including freshwater crayfish may also consume algae; however, fish and crayfish are usually completely excluded from AMD waters.

Of all benthic invertebrates, snails may be among the most important obligate grazers. However, they are unable to tolerate low-pH waters. In a survey of 65 natural and AMD-impacted streams on the West Coast of the South Island of New Zealand, snails were not collected at any site with a pH less than 7 (Harding, unpublished data). In many New Zealand rivers, leptophlebiid mayflies, which also graze algae, may occur at high densities. However, Harding (2006) found that mayflies were almost entirely absent from AMD streams with pH < 4.2 in New Zealand. In contrast, several New Zealand invertebrate taxa are tolerant of
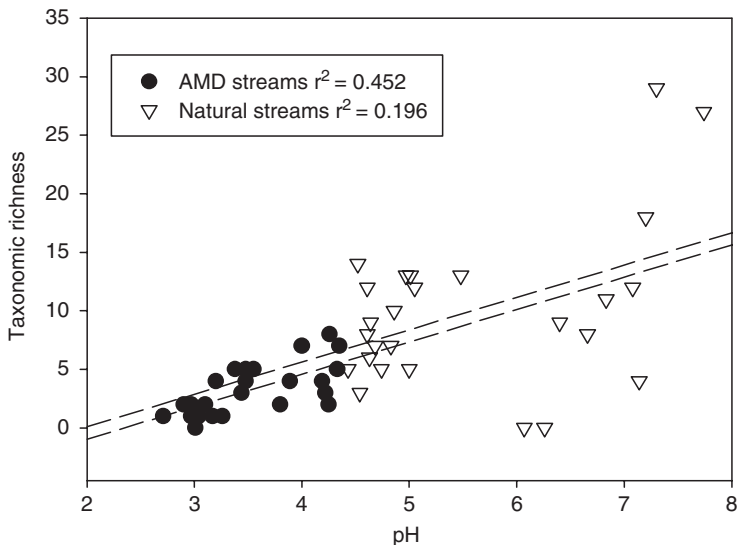
Figure 3. Correlations between benthic invertebrate taxonomic richness and pH in 50 streams on the West Coast of the South Island of New Zealand.

highly degraded AMD, including Chironomidae (nonbiting midges), which occur in streams of pH 2.9 dominated by *Klebsormidium*.

5. Use of Algae to Diagnose and Remediate AMD

5.1. ALGAE AS INDICATORS OF AMD CONTAMINATION

The use of algae as a diagnostic tool for the assessment of AMD requires that test species be sensitive to AMD contamination (e.g., *Pseudokirchneriella subcapitata*, Moreira-Santos et al., 2004; *Selenastrum capricornatum*, LeBlond and Duffy, 2001). The idea of using "indicator organisms" for AMD detection in the field has been advocated by Nakanishi et al. (2004). However, as AMD contamination is frequently obvious or easily measured, the value of these as tools may be limited.

Algae have been used in the assessment of AMD effects by using measures of diversity, abundance, and metal content (e.g., Perrin et al., 1992; Vinyard, 1996; Genter and Lehman, 2000; Winterbourn et al., 2000; Niyogi et al., 2002). These studies typically report an assemblage that is characteristic of AMD-contaminated habitats in the more extreme sites (see Section 2).

5.2. ALGAE AND REMEDIATION OF AMD

Remediation of AMD is extremely challenging (Nordstrom and Alpers, 1999), and usually involves either active or passive treatment systems. Active systems employ the addition of chemicals to contaminated water to reduce acidity and metals, while passive systems often involve the use of wetlands or treatment ponds. Although there is little evidence that algae can raise the pH of AMD, they can certainly remove metals from solution (Stevens et al., 2001). The adsorption of heavy metals is highly variable, depending on the metal, the taxon, age of material, and other conditions. Since inactivated algal biomass also adsorbs metals, a large component of uptake is a passive mechanism of adsorption to cell walls (Chu and Hashim, 2004).

Removal of heavy-metal contamination using algae has been at least partially successful in field trials (e.g., Gale and Wixson, 1979; Phillips et al., 1995; Xie et al., 1996; Van Hille et al., 1999; Kalin et al., 2005). The use of wetlands as an effective remediation solution for AMD is gaining acceptance (e.g., Howard et al., 1989; Dodds-Smith et al., 1995; Jones et al., 1996; Bernoth et al., 2000; Groudeva et al., 2004). The motivation for choosing such a system is often that active treatment such as lime addition, while efficient, becomes expensive over time (Van Hille et al., 1999). However, there is still considerable debate concerning the effectiveness of passive systems (e.g., see http://www.dartmouth.edu/~cehs/CAGsite/docs/report_6.html). Their effectiveness may be limited by topography (e.g., created wetlands require considerable land), the difficulty in optimizing uptake due to field conditions, and reclaiming metals from algal biomass.

In addition to field trials, laboratory experiments have examined the uptake of heavy metals by a number of algal strains. Some freshwater strains used in laboratory studies of metal uptake are given in Table 3. A comparison of these studies with those in Table 2 (taxa reported from AMD-contaminated habitats in the field) reveals that the two lists are almost mutually exclusive. The choice of test organisms in uptake studies may be largely influenced by the availability of cultured strains (many of those in Table 3 are well-known laboratory organisms). Because the pH of AMD may be considerably lower than other types of industrial effluent, it may be difficult to extrapolate the results of these uptake studies to AMD-impacted systems. Several workers reported an optimum pH for uptake of metals of greater than 5.0 (Chen et al., 2005; Gong et al., 2005; Gupta et al., 2006). The optimum pH range for growth of *Chlorella vulgaris* in mixed culture is 6.31–6.84 (Mayo, 1997), similar to that for metal uptake, and the toxicity of heavy metals to *Chlorella* may also be pH dependent (Franklin et al., 2000). Given that pH optima for growth of some acidophilic strains are much lower, further laboratory study of metal uptake by acidophiles is warranted.

Regardless of the limitation of remediation approaches, systems left to recover naturally from AMD contamination show extremely slow rates of rehabilitation. For example, Lake Orijarvi in Finland still has low productivity and lacks a planktonic diatom community, even though mining ceased in 1956

Table 3. Recent studies of heavy metal uptake by freshwater algae.

Algae	Metals	Reference
Scenedesmus obliquus (Turp.) Kütz.	Cr	Pellon et al. (2003)
Microspora sp.	Pb, Ni	Axtell et al. (2003)
S. obliquus, *Cyclotella meneghiana* Kütz.	Ag, Co, Cs, Mn	Adam and Garnier-Laplace (2003)
Spirogyra sp.	U	Aleissa et al. (2004)
Anacystis nidulans Gard.,	Ni, Zn, Cd	Awasthi and Rai (2004)
Chlorella vulgaris Beij.,		
Scenedesmus quadricauda (Turp.) Bréb.		
Spirulina maxima (Setch. & Gard.) Geitler	Pb	Gong et al. (2005)
Nostoc muscorum Ag.,	Cu, Co, Pb, Mn	El-Sheekh et al. (2005)
Anabaena subcylindrica Borge		
Chlamydomonas reinhardtii Dang.	Pb, Cd, Hg	Tuzun et al. (2005)
Microcystis aeruginosa (Kütz.) Elenkin	Pb, Cd, Hg	Chen et al. (2005)
Pseudokirchneriella subcapitata	Cd	Casiraghi et al. (2005)
(Korshikov) Hindák		
Spirogyra sp.	Cu	Gupta et al. (2006)
Synechocystis aquatilis Sauv.	Cu	Ergene et al. (2006)

(Salonen et al., 2006). Streams flowing from mine adits will continue to release AMD into the environment after the mine is abandoned, due to continued oxidation of exposed pyrites (Johnson, 1998).

Studies to date suggest higher potential for remediation of these systems if we can more effectively enlist the help of acidophilic microalgae. Possible strategies include laboratory studies of metal uptake by acidophilic strains to determine optimal conditions, low-cost technology to maintain these conditions in field sites, and techniques to efficiently harvest algal biomass and recover the metals.

6. Summary

AMD creates an extreme water-chemistry environment by drastically lowering the pH of waterways and exposing them to high concentrations of heavy metals. These conditions are most commonly derived from the oxidation of pyrites as a result of mining activities.

A number of algal taxa have been reported from these environments, but a few (e.g., species of *Euglena* and *Klebsormidium*) are far more common than others. These taxa form assemblages that are characteristic of AMD-contaminated ecosystems. However, our understanding of the role of algae in AMD systems is still hindered by issues of nomenclature, uncertain species concepts, and a lack of understanding of the evolution of AMD-tolerant taxa. Despite these issues, molecular studies indicate that AMD-tolerant taxa arise through colonization of AMD-contaminated habitats by local pH-neutral populations, rather than through long-distance dispersal from other areas of the globe. The mechanism of evolutionary change appears to differ between taxa, for example, *E. mutabilis* has

acquired greater heavy metal tolerance than related species, whereas *K. acidophilum* has reduced its pH optimum.

Interactions between different trophic levels in AMD-contaminated systems have been poorly studied. However, biological oxidation of pyrites by bacteria means that the interactions of these organisms affect all other components of the food web. The phagocytotic *E. mutabilis* presumably grazes bacteria, and this may be a key reason for its success in these environments. In turn, bacteria may use algal biomass as a carbon source to power sulfate reduction. In natural stream and river systems, algae form a basal resource for ecosystem food webs; in AMD systems, many benthic invertebrates that normally graze on these algae are absent. Severely impacted AMD systems have highly truncated food webs with few invertebrates, and no higher invertebrates (e.g., crayfish) or vertebrates (e.g., fish).

Several studies have shown that algae can adsorb heavy metals, suggesting their possible use in remediation. However, attempts to turn this observation into remediation technologies have met with mixed success, and require a greater understanding of AMD effects on biota.

7. Acknowledgments

8. References

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