Manganese Removal by the Epilithic Microbial Consortium at Pinal Creek near Globe, Arizona

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ABSTRACT

Interaction of an acidic mine drainage plume with subsurface material in an alluvial aquifer has released dissolved manganese ((Mn(II)) into the perennial reach of Pinal Creek near Globe, Arizona. A combination of hydrologic and biogeochemical precesses is responsible for precipitation of a fraction of the entering Mn(II) as Mn-oxyhydroxides on surficial sediments, within the streambed sediments, beneath algal mats formed on surficial sediments, and among mosses and emergent aquatic plants. This study focuses on the variety and seasonality of biological processes associated with Mn-oxyhydroxide precipitates formed on glass substrates placed in surface waters characterized by different flows and vegetation. The glass slides were emplaced monthly at a single subreach of Pinal Creek to assess epilithic attachment and Mn oxidation; epiphytic oxidation was assessed periodically also. Oxidized Mn was associated with almost every organism in the consortium at Pinal Creek, from the microscopic to the macroscopic. Epilithic bacteria, fungi, algae, and protozoans were coated with oxidized Mn; every macrophyte examined had patches of oxidized Mn. The dominant epilithic precipitation forms were around holdfasts and within secreted substances. The black holdfasts of the iron bacterium, Leptothrix discophora, and the green alga, Ulothrix sp., were doughnut-shaped forms. Expansive patches of black extracellular polysaccharides were secreted primarily by bacterial filaments and fungal hyphae. The dominant macrophytic precipitation form was clumps of oxidized Mn on mosses, green algae, and cyanobacteria. These clumps are consistent with Mn precipitation by elevated pH during photosynthesis. More Mn-oxide precipitates were found in the spring and summer months than the fall and winter, consistent with biological and chemical activity models, and more formed in swifter water than in slower moving water, consistent with oxygen elevation models. These findings provide a better understanding of the biological factors that influence natural attenuation of Mn at Pinal Creek and identify some of the complex interactions between biota, hydrologic processes, and water chemistry that need to be considered to fully assess the affects of acidic mine drainage on stream systems.

INTRODUCTION

Manganese (Mn) is typically released from the rock strata and enters surface and ground water during mining. Mn is dissolved in anoxic and acid water. Homogeneous precipitation of Mn(II) as an oxide phase does not occur below pH 8 but Mn(II) oxidation does occur in the presence of different mineral surfaces and/or via bacterial processes between pH 6 and 8 (Diem and Stumm, 1984). It is also known that bacterially mediated oxidation of dissolved manganese (

Mn(II)) is an important component of metals redox chemistry in soils, and marine and fresh water environments (Ehrlich, 1996). Among the microorganisms capable of oxidizing Mn(II) are bacteria, algae, yeast, and fungi (Ehrlich, 1996). Because aqueous environments are dynamic, and water chemistry, aquatic plants, and algal communities can change with time, even when Mn(II) precipitates as stable oxide phases, these precipitates can be resolubilized if new reducing environments form.

Release of Mn(II) is a major problem for the mining industry. Federal regulations require that Mn(II) released from active mines not exceed 4 milligram per liter (mg L^{-1}) (U.S. Code of Federal Regulations, 1996), a value that is in practice typically far exceeded. Mn(II) is not generally considered a health risk and the main interest has been on the formation of Mn-oxyhydroxide precipitates since other metals (e.g., Zn (zinc), Ni (nickel), and cobalt) can be removed from solution by these precipitates (Hem and others, 1989; Tamura and Furuichi, 1997).

Remediation efforts are underway around the world to utilize chemical, hydrologic, and biological processes to precipitate Mn(II) from acidic mine drainage (AMD). Both active and passive treatments are being explored. Active, and expensive, treatment typically utilizes CaO and NaOH to raise the pH in treatment ponds (Robbins and others, 1997). Municipal water treatment plants in the United States rely on oxidants such as chlorine, while biologically active sand filters

are used in Europe (Vandenabeele and others, 1992). Passive treatments involve the establishment of oxidizing zones in wetlands and limestone beds (Hedin and others, 1996; Watzlaf, 1997). Localities where oxidized Mn is naturally precipitating are being studied intensively (Usui and Mita, 1995) to learn about processes that might be used or modified to apply to AMD contaminated systems and lower treatment costs (Phillips and others, 1995).

Three biological processes are particularly effective in precipitating Mn oxyhydroxides. The neutralophilic iron bacteria are the major microbial group that catalyzes the oxidation of Mn(II) (Nealson, 1982); a process that produces energy for some bacteria, but not all (Ehrlich, 1996). Cyanobacteria and algae have been found to cause precipitation of oxidized Mn as a result of pH elevation due to photosynthesis to values greater than 8 (Richardson and other, 1988). Enzymatic reactions involving extracellular polysaccharides (EPS) may also oxidize Mn (Tebo and others, 1997).

Pinal Creek, near Globe, Arizona, is a locality where oxidized Mn precipitates naturally after partially neutralized, contaminated ground water enters the perennial reach. Geochemical, hydrologic, and biological processes have been invoked to explain both dissolution and precipitation of Mn oxyhydroxides in Pinal Creek. Geochemical mechanisms (Lind, 1991), and hydrologic mixing of surface and ground water in hyporheic zones, coupled with biogeochemical processes within these microbially active zones have been proposed to explain field observations that 20 % of the Mn(II) entering the perennial reach is precipitated over the upper 4 km (kilometer) of the reach (Harvey and Fuller, 1998; Marble and other, 1999a).

This paper focuses on bilogical processes that occur outside of the hyporheic zone in the water column above the streambed sediments. Specifically, we have investigated the wide variety and seasonal changes of biotic processes that are associated directly, or indirectly, with Mn-oxyhydroxide precipitation on surficial sediments and among vegetation present in the perennial reach of Pinal Creek.

STUDY SITE

Pinal Creek is the outlet of the Pinal Creek Basin (a typical alluvial basin of the Southwest) and the perennial stream reach (approximately a 13 km length from head of flow to its confluence with the Salt River) is fed by ground water from the alluvial aquifer that has contaminated by copper-mining activities in the area (Eychaner, 1989; Brown and Favor, 1996). This riparian system has been designated a State of Arizona Superfund site and as a USGS Toxic Substances Hydrology Program site (the USGS has been studying the hydrology, geology and contaminant plume since 1984). Surface water chemistry in the upper 4-km length is dominated by discharge of partially neutralized, metals-contaminated ground water with pH increasing from 5.5-6 at the head of flow to about 8 at the confluence with the Salt River. Manganese remains the primary metal contaminant in the system with lower levels of Zn and Ni (Eychaner, 1989; Gellenbeck and Hunter, 1994; Konieczki and Angeroth, 1997). Mn(II) at the head of flow have been as high as 99 mg L^{-1} (1.8 mM, millimolar) but has remained constant at about 66 mg L^{-1} (1.2 mM) since 1990 (Gellenbeck and Hunter, 1994; Konieczki and Angeroth, 1997; Marble and others, 1999a, 1999b).

Vascular aquatic plants include water speedwell (Veronica anagallis-aquatica) and grasses such as rabbit foot (Polypogon monspeliensis) (Marble and others, 1999b). Spindler and Sommerfeld (1996) found 62 kinds of algae including cyanobacteria, greens, euglenids, cryptomonads, and golden brown algae including diatoms. Bioconcentration of Mn by algae and vascular aquatic plants has been shown to occur (Marble and others, 1999b).

Mixtures of Mn oxyhydroxides of various textures from fine, flocculent materials to layered concretions within the streambed sediments resembling asphalt at some locations are found along the entire reach (Lind, 1991). These precipitates form on top of the sediment bed, within the hyporheic zone (2-20 cm (centimeter) typical thickness, Harvey and Fuller, 1998), among mosses and the root structures of aquatic plants, and beneath algal mats present on sediments and sandbars. The precipitates range from < 75 μm (micrometer) particulate materials to coatings on medium sand and on larger grains.

A subreach that is actively precipitating oxidized Mn in Pinal Creek was chosen for a year-long study between October 1997 and October 1998 (see Figure 1, GPS location 33° 33' 10.41" N lat., 110° 53' 13.67" W long., at an elevation of 850 m (meter)). We conducted are experiments about 20-25 m upstream of site J^2-5 because it is sampled on a regular basis by researchers at the University of Arizona and because it has been a source of biotically active surficial and subsurface sediments that oxidize Mn(II). Analyses of Mn oxyhydroxide-coated sediments collected at this location indicate Mn loadings of 1,000 to 350,000 mg Mn per kg (kilogram) of dry sediment, i.e, 0.1% to 35% by weight Mn (Marble and others, 1999a).

Two locations near opposite banks of the creek were selected because the flow regimes were representative of the extremes found at J^2-5. Analyses of tracer data indicated a main channel flow of approximately 0.18 $m^3 s^{-1}$ (cubic meter per second) which yields a mean velocity of 20-25 cm s^{-1}(per second). Due to the presence of small sandbars (15 cm to 1 m separation) on the left-hand side (LHS) of the creek the water velocity ranges from the main channel value to about one-half this value. As a result of vegetation, and fine sediments trapped by this vegetation, the RHS velocity ranges from about 1 cm s^{-1} to about one- half of the main

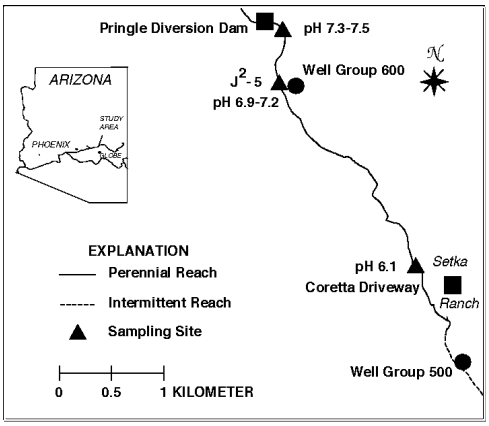

Figure 1. Field site and location in the upper perennial reach.

channel value. Flowrates also change seasonally and episodically due to precipitation at the site or elsewhere in the Pinal Creek Basin. The total braided channel width is 13.2 m, the main channel is 2.2 m in width; and the maximum depth of the channel is 45 cm.

MATERIALS AND METHODS

Biological Analysis

The experiment was designed to learn about biological processes associated with precipitation of xidized Mn on surfaces in the oxic water column above the sediment-surface water interface and on surfaces next to small sandbars and vegetation zones. The primary objective was to analyze epilithic attachment, the process by which organisms colonize and cause precipitation on surfaces. A few samples were analyzed for epiphytic attachment (the process by which organisms colonize and cause precipitation on macrophytes), specifically, on moss, algae, and speedwell. Analysis consisted of detailed microscopic studies of dry slides and moist macrophytes.

Glass microscope slide sets (artificial substrates) were tied to wooden dowels and placed into the creek at 2 sites (2 sets on the LHS, 3 sets on the RHS) and during the next year were replaced monthly to view changes in precipitation of oxidized Mn. When retrieved, slides were rinsed with deionized water to prevent salt crystal growth and allowed to air-dry at the site before returning to the laboratory for analysis. The drying step is important because of prolific growth of an unidentified fungus/actinomycete on the Mn-bearing glass substrates. The presence of oxidized Mn was tested with orthotoluidine (Morgan and Stumm, 1965).

The LHS slide sets were in a narrow channel approximately 1 m from the left bank defined by two small, water speedwell-covered sandbars. The chemical parameters for surface water in this small channel were essentially those of the main channel. The RHS slide sets were in a vegetation zone characterized in the initial months of this study by slower flows than found in the main channel or the LHS sites. This location had a relatively high vegetation density of water speedwell and grasses with fine silts and sands around the submerged plant structures and a substrate of organic-rich material producing local reducing zones.

Water chemistry (Mn(II), pH, dissolved oxygen (O_2), and alkalinity), temperature, and flow characteristics were measured as part of other research projects at Pinal Creek.

RESULTS AND DISCUSSION

Evolution of Site Over Study Year

As the sampling year progressed, changes occurred in the main channel, as well as the LHS and RHS of Pinal Creek. These changes included increased consolidation of the sediment bed (the depth of sediments that could be easily penetrated decreased from 70 cm to between 5 and 20 cm), fill-in due to sediment transport from upstream from rainfall

events, fill-in due to plant succession, and changes in vegetation densities and distributions across the channel. Increases in the density and distribution of water speedwell were observed across the entire channel. The vegetation zone along the RHS increased from 45 cm to between 1 and 1.5 m in width. Algal mats were established on the sediment bed in the main channel and on the sides of the small sandbars. Although normal and episodic rainfall events did not entirely scour out the vegetation (a typical occurrence in the past), fine silt-size sediments were swept into the area periodically and caused the death of a significant fraction of mature water speedwell. Filamentous algae then appeared in the main channel and smaller channels between sandbars and served as anchoring sites for new water speedwell to take root. A layered structure of dead plants and sediments coated with oxidized Mn, and new plant growth on top of this material was observed and reflected this succession of plant types and density.. In addition, the reducing zones on the RHS observed in the fall of 1997 (an odor from sulfur-containing species was released when this organic-matter-rich material was disturbed) decreased in size and disappeared over the course of this study. These changes undoubtedly affected the results of our study.

Although the initial positions of the slides were controlled upon submersion in the system, flow conditions over the period of submersion in LHS often moved both sets of slides into contact with the left bank of the sandbar. In addition, one or both sets of slides were frequently found underneath algal mats that had formed in a one-month period and/or within the root structures of water speedwell not present on the date of submersion. Movement of the slide sets on the RHS were also observed.

During this study year, chemical parameters of surface water in the main channel were pH (6.9-7.2), dissolved O_2 (0.25-0.15 mM), temperature (8-37 °C), alkalinity (40-65 mg L^{-1}), and Mn(II) (47 ± 3 mg L^{-1}, or 0.85 ± 0.055 mM). Mn(II) values peaked in midsummer at 54 mg L^{-1} (Tables 1 and 2). The ranges listed are due to seasonal and diel changes. Shallow groundwater beneath the main channel was sampled once at 70 cm below the sediment-surface water interface with a standard drivepoint sampler 1.2 m in length. The chemical parameter values were pH (6.6), dissolved O_2 (0.033 mM), temperature (15 °C), alkalinity (127 mg L^{-1}), and Mn(II) (6 mg L^{-1} or 0.11 mM); no across stream dependence has been observed. These values for surface and ground water are typical of the water chemistry at J^2-5 before active remediation of the contaminated groundwater plume began in the first part of October, 1998.

Biological Interactions with Manganese in the Zone of Oxidation

Almost every organism at the J^2-5 study site, from microscopic to macroscopic, was associated with precipitates of oxidized Mn. Oxidized Mn coated bacteria, fungi, protozoans, and algae in the epilithic consortium (Table 1). Every macrophyte examined (Table 2) had patches of oxidized Mn, suggesting precipitation of oxidized Mn onto them.

Many iron bacteria are known to co-precipitate Mn (Nealson, 1982), and several of these were sampled as epiliths at Pinal Creek. Oxidized Mn precipitates on epilithic bacteria were predominantly attached to Mn-coated holdfasts anchoring the iron bacterium, Leptothrix discophora, to surfaces, a finding consistent with other research (Robbins and others, 1992; Robbins, 1998). This bacterium was present year round (Table 1) at both the LHS and RHS sites, but oxidized Mn coated bacteria were found in greater numbers in the spring and summer months at the LHS locality (Tables 1a and 1b). This may be due to increased bacterial populations during this period and/or an increased rate of Mn oxidation at more optimal temperatures for biological activity (Tipping, 1984).

Other bacterial morphotypes found with precipitated oxidized Mn on individual cells were cocci, rods, filaments, and a rosette form that is usually called Metallogenium (Maki and others, 1987). Metallogenium was the dominant form found on slide sets placed in freely flowing water (Table 1b), but the Metallogenium-type rosettes at the LHS locality were present only periodically and never abundant. The brown rods were present throughout the year and were common at the LHS site but rarer at the RHS site.

A mixed population was found in contact with vegetation (Tables 1a and 1c) and in the fine sediments associated with the water speedwell root structure at the RHS (Table 1e). This includes Leptothrix discophora filaments, an unnamed Leptothrix that has cells that stay inside the sheaths ("L. inside"), Metallogenium, and Siderocystis. Biofilms of various colors, including the brown color of oxidized Mn, coated microscope slides; biofilms are typically formed by the rods and cocci attached to them (Brown and others, 1994).

Since fungi and actinomycetes are known to precipitate Mn (the American Type Culture Collection, 1996; lists 4 species of fungi), it was not unexpected that some evidence of this process would also be found at Pinal Creek. Precipitated oxidized Mn was found only on the hyphae of epilithic fungi and actinomycetes (Table 1); precipitates were not found on spores. This type of precipitation occurred on all slide sets at both emplacement sites, but mainly at LHS. Oxidized Mn took two forms: smooth patches along some hyphae, and loose, rather lumpy accumulations on other hyphae. At present, the dominant fungus has not been identified, but it has elongated, segmented colorless hyphae that colonize entire surfaces of the slides; its colorless spores resemble those of actinomycetes. Its sporangia are also colorless. This unidentified fungus

became so heavily coated that a thick, black sooty oxidized Mn powder formed on LHS-V1 (#101) and RHS-V1 (#65) slides.

On certain slides as much as 50 % of the Mn precipitates were associated with epilithic algaes. The dominant type was coated holdfasts of the filamentous green alga, Ulothrix. Spindler and Sommerfeld (1996) identified 2 species of Ulothrix in Pinal Creek, both present at the current study site and both forming holdfasts. The Mn-coated holdfasts were primarily found at the LHS site and were most abundant in August at the LHS-V1 slow flow site; they were abundant during many months at the LHS-V2 moderate flow site. Smooth accumulations of precipitated oxidized Mn. were noted on filaments of Ulothrix, Spirogyra, and an unidentified filamentous green alga. Lumpy accumulations built up in patches of intertwined filaments. This type of oxidized Mn precipitate is similar to that ascribed to localized photosynthetic elevation of pH (Richardson and others (1988).

Other algae and cyanobacteria not yet identified are most probably involved in precipitation of oxidized Mn, as mixed colonies have been found to be most efficient (Phillips and others, 1995; Stuetz and others, 1996). In addition, changes in populations and age will influence the role these microorganisms have since it has been shown that cyanobacteria and algae must attain a certain size before Mn is oxidized on the cells (Richardson and Stolzenback,1995).

In some instances, epilithic diatoms were coated brown from oxidized Mn. Hunt and Smith (1980) recognized that diatoms precipitated Mn but were unsure of the mechanism. If photosynthesis alone were sufficient to explain the association of oxidized Mn precipitates with diatoms, then every diatom in the Pinal Creek data set should have been coated brown, which is not the case.

Oxidized Mn was most commonly observed on EPS at Pinal Creek. EPS and Mn were concentrated on the external cell walls and holdfasts of bacteria, hyphae of fungi, and on the filaments of algae, and decreased with distance away from these structures. EPS around these structures was present at all sampling localities, but more abundant at the LHS site. The moderate flow site at LHS had abundant EPS in the spring months, but even the slow flow and no flow sites at RHS had significant accumulation by EPS in the summer months.

This visual inspection suggests that EPS secreted by cells, holdfasts, hyphae, and filaments plays a key role (direct and/or indirect) in oxidation of Mn(II) at Pinal Creek. This inference receives support from studies that indicate that Mn oxidation by bacteria takes place within a complex matrix of excreted heteropolysaccharides (Tebo and others, 1997). Additional support comes from work reported by Fortin and others (1997), who proposed an indirect, non-enzymatic mechanism for Mn oxidation in which EPS simply serves as sorption and nucleation sites.

Epilithic protozoan cells and tests were patchily coated by oxidized Mn. In most instances, bacteria had colonized the cells and tests and precipitated the Mn, but in some cases it appeared that the Mn precipitated directly on the protozoan cells and tests.

Macrophytes were also found with oxidized Mn precipitates (Table 2). Much of the Mn was attached to holdfasts of Leptothrix discophora and Ulothrix that colonized the mosses and speedwell, but clumps that are similar to those ascribed to pH elevation during photosynthesis were noted on mosses, speedwell, and cyanobacteria.

Biological Interactions with Manganese in the Zone of Weak Oxidation/Reduction

There is a distinct difference between the numbers and types of organisms found on the glass substrates emplaced in the moderate and slow flow of LHS versus the highly vegetated RHS site having very slow, or no perceptible, flow. The RHS sets, like the LHS site, had epilithic bacteria, fungi, algae such as Ulothrix and diatoms, and protozoans. However, filamentous bacteria on the RHS were more diverse than from the LHS site, and the RHS sets had either significantly less or no oxidized Mn.

The glass substrates were pushed into the vegetation and fine sediments and/or organic-rich substrate at RHS to sample the zone of reduction and the redox zone. In only one instance was the redox zone unequivocally captured. At RHS-V1 between September to October, slides #105 and 106 had a distinct red/orange line that identified the location of the iron redox zone at the sediment-water interface. The biofilm above this zone was brown in color, contained medium and short rods, and tested positive for oxidized Mn. This biofilm contained red bacterial filaments and sheaths, numerous diatoms, and red-coated short and medium rods. Below this, a biofilm was lacking and the attached bacteria were all colorless.

CONCLUSIONS

Organisms appear to have a major role in precipitating oxidized Mn on artificial substrates emplaced at the Pinal Creek study site. Oxidized Mn was found on bacteria, cyanobacteria, algae, fungi/actinomycetes, protozoans, and macrophytes and the macroscopic appearance of the precipitates often resembled that of the specific organism. Most of the

oxidized Mn was observed on holdfasts and EPS. Holdfasts with oxidized Mn pesent include those of the green alga, Ulothrix, and the iron bacterium, Leptothrix discophora. EPS and oxidized Mn were found around bacterial cells and holdfasts, fungal hyphae, and algal holdfasts and filaments.

Our study indicates that there is a seasonal component to the precipitation of oxidized Mn, since the glass substrates were more heavily coated with oxidized Mn during the spring and summer months. Whether this is due to a dependence of biological activity on temperature (population types, numbers and density), or a temperature dependence of an underlying chemical mechanism, cannot be answered with the current data, but it is a question that can be addressed in future research.

The observation that more Mn precipitated onto the glass substrates where water flow was fastest may reflect a dependence on velocity of mass transfer of key chemical species from the bulk surface water to the local chemical environments associated with the biofilm communities responsible for precipitates of oxidized Mn. Transport of nutrients, dissolved O_2, and Mn(II) to active sites would certainly be expected to depend on water velocity but confirmation of this inference would require measurements at the microscale.

There are indications that some of the oxidized Mn precipitates may be easily reduced or mobilized in response to stream water chemistry changes. For example, the sooty form of oxidized Mn on the unidentified fungus/actinomycete appears to be very loosely bound to surfaces; physical detachment of the anchoring structures may be a facile process occurring in response to relatively small changes in pH and/or major ions. This could be readily tested in the laboratory. The stability of different oxidized Mn precipitates could be tested in the field by placing colonized glass substrates into reducing zones to learn which form of biologically precipitated oxidized Mn lasts the longer.

Results of this year-long study indicate that a consortium of microorganisms at Pinal Creek acts in concert to remove Mn(II) from circumneutral surface water. Both bacteria (e.g., Leptothrix discophora) and algae (e.g., Ulothrix sp.) are associated with this overall process, with EPS having some general, but not yet defined, role. The positive roles of temperature and water velocity in increasing Mn(II) removal by microorganisms is suggested by our data and Ulothrix sp. has been identified as a particularly effective organism for removal of Mn(II). However, quantitative studies must be undertaken (field and laboratory) to define the contribution of the epilithic communities to overall Mn(II) removal and the environmental conditions that optimize this overall process. It may then be possible to suggest constructive ways in which to use these natural attenuation mechanisms in remediation efforts at different contaminated sites.

ACKNOWLEDGMENTS

This publication was made possible by grant number P42 ESO4940 from the National Institute of Environmental Health Science with funding provided by EPA and by grant number EAR-95-23881 from the NSF. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS, NIH, or EPA, or NSF.

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Table 1a. **Chemical and microscopic biological data at site LHS-V1 [slides in contact with vegetation]**
(Symbols and abbreviations: +++++, >5 every field; ++++, >2 every field; ++, 1 every field; +, <1 every field; ---, not present or no data; c, curved; co, cocci; l, long; m, medium; r, rod; s, short; vi,vibrio)

No.	Submersion dates	Temperature (ºC) and season at retrieval date	pH at retrieval date	DO (mg/L) at retrieval date	Mn(II) (mg/L)	Brown cocci (co) or rods (r) (l,long s,short)	Brown coated bacterial filaments	Iron bacteria	Brown holdfasts of Leptothrix discophora (or other)	Colored biofilms	Brown exo-polysac-charide	Brown coated fungal hyphae	Brown algal holdfasts (and filaments)	Diatoms (*brown coated) (no. of species)
1-2	10/6/97-11/17/97	14.3 Fall	7.06	---	46	---	---	---	+++	---	+	---	+	+* (4)
11-12	11/17/97-12/17/97	--- Winter	---	---	45	+ (sr)	+	---	+ (+ other)	---	+	+	+	+ (7)
19-20	12/17/97-1/20/98	13.8 Winter	6.94	---	45	---	---	Siderocystis	+	---	---	---	+	+ (6)
33-34	2/20/98-3/19/98	18.4 Winter	---	---	47	----	---	---	+ (+ other)	brown (sr)	+	---	+	+ (4)
43-44	3/19/98-4/17/98	18.5 Spring	7.08	---	43	---	---	L. discophora	+++++	---	---	---	++	+ (4)
51-52	4/17/98-5/18/98	20.2 Spring	---	---	44	+	---	---	++++ (+ other)	---	+++	+	+	+ (5)
61-62	5/18/98-6/24/98	--- Summer	---	---	---	---	+	L. discophora	++++ (+ other)	orange	+	---	+	+ (7)
71-72	6/24/98-7/22/98	22.7 Summer	7.41	---	54	++ (sr,cr)	++	---	+	brown (sr)	+++	+	++	+* (7)
81-82	7/22/98-8/21/98	25.6 Summer	7.10	7.3	50	+ (sr,co)	+	+Metallogenium?	+++++	---	+++	+	+++++	+* (8)
91-92	8/21/98-9/29/98	--- Summer	---	---	47	+ (sr,vi)	+	+ Metallogenium	+	beige (sr)	+	---	+	++ (5)
101-102	9/25/98-10/30/98	16.6 Fall	6.76	8.4	36	+ (sr, lr)	+	++Metallogenium	+	yellow, orange (sr)	+	+	+ +filaments	++* (11)

Table 1b. Chemical and microscopic biological data at site LHS-V2 [slides freely floating in water moving 20-25 cm s^{-1}] (See Table 1a for symbols and abbreviations)

No.	Submersion dates	Temperature (°C) and season at retrieval date	pH at retrieval date	Mn(II) (estimated) mg/L	Brown cocci (co) or rods (r) (l,long s,short)	Brown coated bacterial filaments	Iron bacteria	Brown holdfasts of Leptothrix discophora (or other)	Colored biofilms	Brown exo-polysac-charide	Brown coated fungal hyphae	Brown algal holdfasts (and filaments)	Diatoms (*brown coated) (no. of species)	Brown-coated proto-zoans
5-6	10/6/97-11/17/97	--- Fall	---	46	---	+	---	+++++	brown (co)	+	---	+++++	+ (5)	+
13-14	11/17/97-12/17/97	--- Winter	---	45	---	---	+ Metallogenium	++	---	+	---	++ (+filaments)	+* (6)	---
21-22	12/17/97-1/20/98	--- Winter	---	45	---	+	---	+ (+ other)	---	+	+	++	+ (5)	---
27-28	1/20/98-2/20/98	--- Winter	---	---	+ (r)	+	---	+	brown (sr)	---	+	++	+ (3)	+
35-36	2/20/98-3/19/98	--- Winter	---	47	---	---	---	+	orange (sr)	---	---	---	+ (6)	---
45-46	3/19/98-4/17/98	--- Spring	---	43	+++++ (r)	+	---	+++++	---	+++++	+	+++++ (+filaments)	+ (3)	---
53-54	4/17/98-5/18/98	--- Spring	---	44	---	+	---	+++++	very dark brown	+++	---	++++ (+filaments)	+* (6)	+
63-64	5/18/98-6/24/98	--- Summer	---	---	+ (mr)	---	---	+++++	brown (sr)	+++++	+	+++++	++ (6)	---
73-74	6/24/98-7/22/98	22.7 Summer	7.43	54	+ (sr)	+	---	+++ (+ other)	brown (sr)	+++	+	+++ (+filaments)	+++++ (8)	---
83-84	7/22/98-8/21/98	--- Summer	---	50	+ (sr)	+	+ Metallogenium	+	brown (co)	++++	+	---	++++* (5)	---
93-94	8/21/98-9/29/98	--- Summer	---	47	+ (sr)	---	---	+	light brown (sr)	---	---	+	+ (6)	---
103-104	9/25/98-10/30/98	--- Fall	---	36	+ (sr,co, lr)	+	+ Metallogenium	+++	---	+++	+	+++ (+filaments)	++++* (9)	---

Table 1c. Chemical and microscopic biological data at site RHS-V1 [slides in contact with grasses, flow ≈cm s^{-1}] (See Table 1a for symbols and abbreviations)

No.	Submersion dates	Temperature (°C) and season at retrieval date	pH at retrieval date	DO at retrieval date (mg/L)	Mn(II) (estimated) mg/L	Brown cocci (co) or rods (r) (l,long s,short)	Brown coated bacterial filaments	Iron bacteria	Brown holdfasts of Leptothrix discophora (or other)	Colored biofilms	Brown exo-polysac-charide	Brown coated fungal hyphae	Brown algal holdfasts	Diatoms (*brown coated) (no. of species)	Brown-coated proto-zoans
3-4	10/6/97-11/17/97	--- Fall	---	---	38	+ (lr)	+	L. inside	+	brown (sr)	+	+	+	+* (5)	+
15-16	11/17/97-12/17/97	--- Winter	---	---	38	---	---	L. discophora	+	---	---	---	+	+ (4)	+
23-24	12/17/97-1/20/98	--- Winter	---	---	38	---	---	---	+	---	---	+	---	+ (2)	---
37-38	2/20/98-3/19/98	--- Winter	---	---	39	---	---	---	+	beige (sr)	---	---	++	+* (7)	---
47-48	3/19/98-4/17/98	--- Winter	---	---	36	---	+	---	---	---	---	---	++	+ (2)	---
55	4/17/98-5/18/98	--- Spring	---	---	37	---	---	---	+ (+ other)	light brown (sr)	---	---	+	+ (3)	---
65	5/18/98-6/24/98	21 Summer	6.7	6.5	---	+ (r)	+	Metallogenium	+	---	++	+	+	+* (10)	---
75-76	6/24/98-7/22/98	23.6 Summer	7.24	---	45	+ (sr)	+	L.discophora, Siderocystis	+	brown (mr,cr)	+++	+	+	+ (4)	---
86	7/22/98-8/21/98	21.6 Summer	6.69	6,5	42	---	---	---	+	---	---	+	---	+ (4)	---
95-96	8/21/98-9/29/98	--- Summer	---	---	39	---	---	L. inside	+	brown (sr, mr)	---	---	+	+ (7)	---
105-106	9/25/98-10/30/98	--- Fall	---	---	30	---	+	L. discophora?	---	yellow, brown (mr)	---	+	+	++ (5)	---

Table 1d. Chemical and microscopic biological data for site RHS-V2 [slides partly in fine sediment; 10 cm downstream from RHS-V1] (See Table 1a for symbols and abbreviations)

No.	Submersion dates	Season at retrieval date	Mn(II) (estimated) mg/L	Brown holdfasts of Leptothrix discophora (or other)	Brown cocci (co) or rods (r) (l,long s,short)	Brown coated bacterial filaments	Iron bacteria	Brown holdfasts of Leptothrix discophora (or other)	Colored biofilms	Brown exo-polysac-charide	Brown coated fungal hyphae	Brown algal holdfasts	Diatoms (* brown coated) (no. of species)	Brown-coated proto-zoans
7-8	10/6/97-11/17/97	Fall	38	+ (+ other)	+ (sr)	+	---	+ (+ other)	light brown (sr)	---	---	+	+ (5)	+
17-18	11/17/97-12/17/97	Winter	38	+	---	+	L. discophora	+	light brown (sr)	---	+	+	++ (8)	---
25-26	12/17/97-1/20/98	Winter	38	---	+ (sr)	---	---	---	orange	---	---	---	+ (10)	---
29-30	1/20/98-2/20/98	Winter	---	+ (+ other)	+ (sr)	+	---	+ (+ other)	brown (sr)	---	---	---	+* (8)	+
39-40	2/20/98-3/19/98	Winter	39	(+ other)	---	---	---	(+ other)	brown (sr)	+	---	++	+ (2)	---
49-50	3/19/98-4/17/98	Spring	36	+	---	+	---	+	brown (mr)	---	---	---	---	---
57-58	4/17/98-5/18/98	Spring	37	+	---	+	L. discophora?	+	brown, red	---	---	+	+ (6)	---
67-68	5/18/98-6/24/98	Summer	---	+	---	---	---	+	---	++	+	+	+++ (6)	---
77-78	6/24/98-7/22/98	Summer	45	+	---	---	---	+	light brown (sr, cr)	---	---	+	+ (2)	---
87-88	7/22/98-8/21/98	Summer	42	+	+ (co)	+	---	+	---	+	+	+	+ (8)	---
97-98	8/21/98-9/29/98	Summer	39	---	---	---	---	---	orange brown (sr)	---	---	---	+ (6)	---
107-108	9/25/98-10/30/98	Fall	30	+	+ (sr)	+	L. discophora	+	brown (sr)	---	---	+	+ (7)	---

Table 1e. Chemical and microscopic biological data for site RHS-V3 [slides pushed entirely into sediments] (See Table 1a for symbols and abbreviations)

No.	Submersion dates	Season at retrieval date	Mn(II) (estimated) mg/L	Brown coated bacterial filaments	Iron bacteria	Brown holdfasts of Leptothrix discophora (or other)	Colored biofilms	Brown exo-polysac-charide	Brown coated fungal hyphae	Brown algal holdfasts (and filaments)	Diatoms (number of species)	Brown-coated proto-zoans
9-10	10/6/97-11/17/97	Fall	38	+	Siderocystis	+	light brown (co, sr)	---	---	---	+ (8)	+
31-32	1/20/98-2/20/98	Winter	---	---	L. discophora, L. inside	+ (+ other)	---	---	---	---	+ (6)	+
41-42	2/20/98-3/19/98	Winter	39	---	L. inside, L. discophora	---	orange (mr,lr)	---	---	---	---	---
59-60	4/17/98-5/18/98	Spring	37	---	L. inside	+	yellow	+	+	---	+ (2)	---
69-70	5/18/98-6/24/98	Summer	---	+	---	++	brown (sr)	---	---	++++	++++ (4)	---
79-80	6/24/98-7/22/98	Summer	45	---	L. discophora?	(+ other)	brown (mr)	---	---	---	+ (4)	---
89-90	7/22/98-8/21/98	Summer	42	+	---	+	light & dark brown (co)	+++	+	+++++ (+filaments)	---	---
99-100	8/21/98-9/29/98	Summer	39	---	---	+	light brown (sr)	---	---	+	+ (3)	---
109-110	9/25/98-10/30/98	Fall	30	+	---	+	yellow, light brown (sr)	---	---	+ (+filaments)	+ (5)	---

Table 2. Physical, chemical, and biological data for macrophytes at LHS (See Table 1a for symbols and abbreviations)

No.	Material	Date	Temperature (°C) and season	pH	DO (mg/L)	Mn(II) (mg/L)	Attached brown Leptothrix discophora holdfasts	Brown clumps
A	Moss	3/19/98	18.4 Winter	---	---	47	+	+
B	Speedwell (Veronica) roots	3/19/98	18.4 Winter	---	---	47	+	+
C	Spirogyra	7/22/98	22.7 Summer	7.4	---	54	+	+
D	Mixed mat of moss and Spirogyra	7/22/98	22.7 Summer	7.4	---	54	+	+
E	Mixture of moss, filamentous green, and Spirogyra	9/25/98	--- Summer	---	---	47	+	+
F	Ulothrix dominantly	10/30/98	16.6 Fall	6.8	8.4	36	+	+
G	Ulothrix and filamentous cyanobacteria	10/30/98	16.6 Fall	6.8	8.4	36	+	+