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Metal Interactions with Microbial Biofilms in Acidic and Neutral pH Environments

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Microbial biofilms were grown on strips of epoxy-impregnated filter paper submerged at four sites in water contaminated with metals from mine wastes. At two sample stations, the water was acidic (pH 3.1); the other sites were in a lake restored to a near neutral pH level by application of a crushed limestone slurry. During a 17-week study period, planktonic bacterial counts increased from 10^1 to 10^3 CFU/ml at all sites. Biofilm counts increased rapidly over the first 5 weeks and then leveled to 10^4 CFU/cm^2 in the neutral pH system and 10^3 CFU/cm^2 at the acidic sites. In each case, the biofilms bound Mn, Fe, Ni, and Cu in excess of the amounts adsorbed by control strips covered with nylon filters (pore size, 0.22 μm) to exclude microbial growth; Co bound under neutral conditions but not under acidic conditions. Conditional adsorption capacity constants, obtained graphically from the data, showed that biofilm metal uptake at a neutral pH level was enhanced by up to 12 orders of magnitude over acidic conditions. Similarly, adsorption strength values were usually higher at elevated pH levels. In thin sections of the biofilms, encapsulated bacterial cells were commonly found enmeshed together in microcolonies. The extracellular polymers often contained iron oxide precipitates which generated weak electron diffraction patterns with characteristic reflections for ferrihydrite ($Fe_2O_3 \cdot H_2O$) at *d* equaling 0.15 and 0.25 nm. At neutral pH levels, these deposits incorporated trace amounts of Si and exhibited a granular morphology, whereas acicular crystalloids containing S developed under acidic conditions.

The behavior of dissolved metals in natural bodies of water is strongly influenced by particulate inorganic and organic material (23, 39). Hydrous metal oxides (31, 38), clays (35), humic substances (22, 26, 36), and biota (25, 28) are all capable of binding metallic ions from solution. This effectively enhances the partitioning of metals into sediments and contributes to authigenic mineral formation during the course of diagenesis (16, 33). Of the various metal-complexing agents in aquatic systems, microorganisms and their constituent polymers are among the most efficient scavengers of metallic ions. For example, planktonic microbial forms have been implicated in metal (i.e., Fe and Mn) uptake within horizontally advected hydrothermal plumes along the East Pacific Rise and southern Juan de Fuca Ridge (10, 11). Similarly, soluble metals are bound by the capsular material which often surrounds bacteria associated with sedimenting colloidal particles in freshwater lakes (32). Certainly, a few specific natural microbial metal interactions (i.e., those of the iron- and manganese-depositing bacteria) have been well studied (19, 20), but unfortunately most of our information concerning general microbial immobilization of metals comes from short-duration laboratory experiments on isolated bacterial cell walls and extracellular polymers (3, 37). For this reason, it is important to develop a better understanding of the reactivity between metallic ions and natural microbial populations.

The formation of nontransient adherent communities (i.e., biofilms) of microorganisms on submerged surfaces is common in both freshwater and marine environments (17, 18, 30, 41). These attached microbial populations benefit from the enriched nutrient status of solid-liquid interfaces and may be more active in terms of metabolic activity than their planktonic counterparts (15). Previous studies have used microbial surface colonization for in situ growth (6), nutrient uptake (29), and biomass determinations (34). A similar approach is taken in this investigation on interactions between metals and microorganisms in their native environment. Biofilms were grown on strips of epoxy-impregnated filter paper strips submerged in water contaminated with metals from mine wastes. At regular intervals over a 17-week study period, samples were analyzed for adsorbed metal and examined by electron microscopy. The results showed that microbial biofilms are not only capable of binding significant quantities of metallic ions under natural conditions but they also serve as templates for the precipitation of insoluble mineral phases.

MATERIALS AND METHODS

Site description. Metal adsorption studies by adherent microbial populations were conducted within acidic and neutral pH environments of the Moose Lake watershed at Onaping, northwest of Sudbury, Ontario, Canada. Above Moose Lake, mine drainage and casual water seep through waste rock and then pass over and through two mill tailing ponds, one of which is still active (Fig. 1). Acid conditions are generated in these areas by the oxidation of pyrrhotite (FeS_{1+x}) and other metal sulfides present in the mine wastes. The acidic metal-laden waters, some of which pass through Cranberry Lake, eventually collect in Upper Moose Lake. Upon entering Lower Moose Lake, the water is treated with a crushed limestone slurry to restore circumneutral pH

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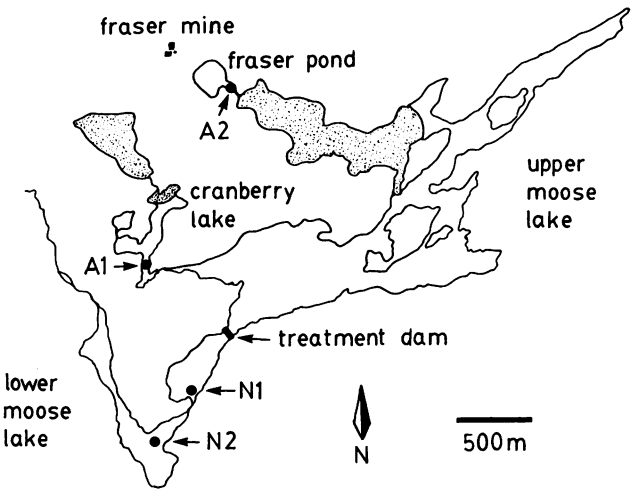

FIG. 1. Map of Moose Lake area showing location of sample sites, Fraser mine, treatment dam, and tailing impoundments (shaded areas).

conditions and reduce downstream dissolved metal concentrations.

Sample stations were established at four sites in the last week of April 1987 (Fig. 1). Anchored buoys were placed in the south end of Cranberry Lake (site A1) in 6.0 m of water and near the discharge of Fraser Mine Pond (site A2) at a depth of 3.0 m. The pH level at both sites was 3.1 and remained constant over the sampling season. Similarly, buoys were anchored in areas of Lower Moose Lake 5.0 to 6.0 m deep, above (site N1) and below (site N2) a narrow channel separating two arms of the lake. At these sites, the pH level was more variable but stayed within a range of 6.5 to 6.9. Also, during the first 8 weeks, surface water temperatures at the four sample stations increased from near 8°C to a stable level of approximately 15°C. Temperature and pH levels were determined whenever samples were collected (see below).

Sampling procedures. Strips of Whatman no. 1 filter paper (Fisher Scientific Co.) were impregnated with an epoxy resin (Epon 812; CanEM), cured, and mounted in plastic Gepe 35-mm photographic slide binders (5.0 × 5.0 cm). Control slides were prepared by sandwiching the support strips between 0.22-μm Nylon 66 micron filters (Schleicher and Schuell, Inc.) to inhibit microbial penetration to the support strips. A UV laminar flow cabinet was used at all times to reduce exogenous contamination of the slides. Test and control slides were threaded onto separate lines attached firmly to weighted plastic hangers. These were subsequently suspended 1.5 m below the anchored buoys at each sample station.

After an initial in situ incubation of 1 week and at approximately 5-week intervals thereafter, paired sets of slides were carefully recovered from each of the sample stations. In each case, the slides were aseptically placed into sterile petri plates for immediate transport to a field laboratory. At the same time, water samples were collected with 4.0-liter acid-leached and autoclaved polypropylene bottles. All samples were prepared for analysis within an hour of being collected.

Enumeration of bacteria. Sterile scalpels were used to cut three 1.0-cm^2 samples from the test and control strips. Of these specimens, two were retained for metal analyses and electron microscopy; the remaining squares were transferred directly into sterile tubes containing 4.0 ml of M-9 salts (GIBCO) adjusted to pH 5.0 or 7.0 for the acidic and neutral pH stations, respectively. A combination of mechanical disruption with a sterile stainless steel spatula, homogenization in a water bath sonicator, and vortexing was used to disperse attached bacteria. By phase microscopy, we could not see any bacteria remaining attached to the support strips. After serial dilution of each suspension in M-9 salts, plates were prepared in duplicate with minimal medium (M-9 salts supplemented with 1.0% [wt/vol] glucose and 0.5% [wt/vol] yeast extract) adjusted to the appropriate pH level. Dilutions of each water sample were also prepared to enumerate planktonic bacteria. After incubation at 20°C for 3 weeks, all plates with fewer than 300 colonies were counted to estimate viable heterotrophic bacterial population density values. In addition, Gram stains of cells from individual colonies were made to determine the distribution of attached or planktonic gram-positive and gram-negative bacteria.

Metal analyses. Specimens from both control and test strips were placed into 30-ml acid-leached polypropylene bottles containing 1.0 ml of 2.0% (vol/vol) HNO_3 (Fisher). Each square was leached for 2 weeks at 20°C before dilution to 10.0 ml with high-resistance (ca. 5 MΩ/cm) deionized distilled water. Water samples were also transferred to polypropylene bottles and acidified with nitric acid to a final concentration of 0.2% (vol/vol). A Perkin-Elmer model 2280 atomic absorption spectrometer operating in the graphite furnance mode was used to determine sample concentrations of Mn, Fe, Co, Ni, and Cu. The instrument was routinely calibrated with corresponding J. T. Baker metal standards.

Data analysis. Biofilm metal adsorption isotherms were graphically constructed by using an empirically derived version of the James-Healy model for metallic ion interactions at solid-liquid interfaces (24). The original equation takes the form of a Freundlich isotherm and relates metal adsorption to electrostatic surface potential. Under conditions of constant ionic strength, surface potential varies directly with charge density which, in a biofilm, should be proportional to the number of attached cells. If ionic strength is assumed to be constant, a logarithmic relationship between biofilm metal adsorption and population density can be established; $\log \Gamma_i = 1/\theta_i \log[\mathrm{CFU}] - pX_i$, where Γ_i is micromoles of metal i bound per cm^2, θ_i is an index of the adsorption strength, [CFU] is CFU per cm^2 (an estimate of the total number of attached cells), and X_i is a conditional adsorption capacity constant expressed as micromoles of metal i adsorbed per CFU. The biofilm metal adsorption data were fit to this equation by linear least-squares regression analysis.

Electron microscopy. Thin wedges cut from the specimen squares were transferred to polypropylene tubes containing 1.0% (vol/vol) glutaraldehyde (CanEM) in water from the corresponding sample stations. In this way, the attached microorganisms were fixed for electron microscopy under conditions similar to those experienced in situ. The samples were subsequently dehydrated through an ethanol-propylene oxide series (both chemicals were from Fisher) and embedded in Epon 812 (CanEM). A Reichert-Jung Ultracut E ultramicrotome was then used to cut sections of ca. 150-nm thickness perpendicular to the wedge surfaces. Duplicate sets of thin sections were prepared from each specimen and mounted on Formvar carbon-coated copper or aluminum grids; one of the grids was routinely stained with uranyl acetate and lead citrate. The unstained grids were imaged directly in the electron microscope relying on the metals complexed in the native environment for electron contrast.

The thin-sectioned specimens were examined with either a Philips EM 300 at 60 kV or a Philips EM 400T at 100 kV. The EM 400T was equipped with a scanning transmission electron microscope unit, goniometer stage, and an EDAX energy-dispersive X-ray spectrometer interfaced to a Tracor Northern series 5500 multichannel analyzer. Both electron microscopes were operated with liquid nitrogen-cooled anticontamination devices in place at all times. Energy dispersive X-ray spectroscopy was conducted by using electron beam spot sizes of 200 nm or less, and spectra were obtained by collecting counts for 100 s (live time). In some samples, selected area electron diffraction was used to examine mineral precipitates. The *d*-spacing of selected-area electron diffraction patterns was calculated with evaporated aluminum as a standard for camera-length calibrations.

RESULTS

Biofilm growth. The planktonic and adherent heterotrophic bacterial population density values for each sample station are plotted in Fig. 2. At each site, there was a significant but variable increase in cell counts over the study period. In contrast, bacteria were not detected on any of the control support strips (i.e., those covered with 0.22-μm-pore nylon filters) until week 11. The numbers of bacteria on the controls at that time and after the last sampling were 10- to 100-fold lower than corresponding values from the freely exposed test strips (data not shown).

Of the four sample stations, acidic site A1 in Cranberry Lake (Fig. 2C) had the highest initial counts of both planktonic and attached bacteria. This observation suggests that the concentration of free-living bacteria plays an important role in determining the initial rate of microbial surface colonization. After week 1, however, the growth rates of the attached bacterial populations appeared to be independent of their planktonic counterparts. The curves for the adherent bacteria were generally hyperbolic as expected for systems controlled primarily by cellular adsorption and growth, coupled with a concomitant emigration of daughter cells (6, 27). In contrast, the free-living bacteria exhibited sinusoidal population curves which are more characteristic of microbial population responses to seasonal warming.

The general pattern of biofilm growth was the same for the two neutral pH sites (Fig. 2, A and B). After a rapid increase in cell numbers over the first 5 weeks, the biofilm growth rate decreased progressively until the last sampling when a population densty of 2.0×10^4 CFU/cm^2 was recorded. Similarly, the numbers of attached bacteria at the acidic stations increased in parallel with one another (Fig. 2, C and D). However, a marked decrease in growth rate was observed after week 5, with final population density values reaching levels of only 4.0×10^3 CFU/cm^2. The lower numbers of attached bacteria found at the acidic sites

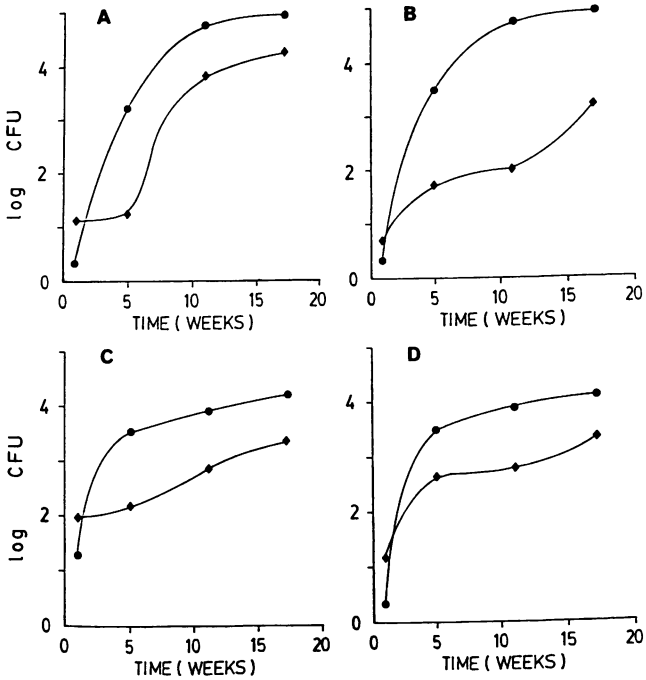

FIG. 2. Growth curves for attached (log CFU per cm^2 [●]) and planktonic heterotrophic bacteria (log CFU per milliliter [◆]) at neutral pH sites N1 (A) and N2 (B) and at acidic sites A1 (C) and A2 (D).

suggest that attachment and growth are stressed by low pH levels and possibly by high dissolved metal concentrations.

Direct examination of all biofilms by phase-contrast light microscopy revealed small ocherous precipitates (>1.0 μm in diameter) and some remnants of diatom frustules. Distinct bacteria were difficult to directly image against the background of the epoxy-impregnated strips. At the neutral pH stations, filamentous cyanobacteria were occasionally observed with rarely more than a single filament present in one field of view. Eucaryotic algae were not observed in these samples; however, small motile unicellular algae (possibly *Chlamydomonas* species) were found on some samples from the acidic sites. Periphytic grazing protozoans were not encountered on any of the biofilm strips. Gram stains of cells from individual bacterial colonies randomly selected from the dilution plates from each site throughout the season (both in the water and on the biofilm strips) revealed that gram-negative bacteria accounted for 75 to 80% of the bacteria.

Metal retention. The mean aqueous metal concentration values for each sample station over the 17-week study period

TABLE 1. Mean aqueous metal concentrations for the sample stations over the 17-week study period

Station	Mean concentration ± SD (μg/ml [ppm])[a] of:				
	Mn	Fe	Co	Ni	Cu
N1	0.65 ± 0.14	2.71 ± 0.67	0.08 ± 0.02	2.10 ± 0.30	0.02 ± 0.01
N2	0.52 ± 0.16	12.56 ± 5.41	0.05 ± 0.04	2.08 ± 0.46	0.02 ± 0.01
A1	1.64 ± 0.02	37.23 ± 15.81	0.29 ± 0.11	5.57 ± 0.72	0.21 ± 0.11
A2	3.57 ± 0.60	62.50 ± 22.62	5.76 ± 2.76	62.92 ± 30.87	1.74 ± 0.65

[a] Figures represent an average of at least five determinations for each sample plus or minus the standard deviation of the mean (ppm, parts per million).

TABLE 2. Amount of metal adsorbed by control surfaces and biofilms after 17 weeks of in situ incubation

Sample[a]	Mean amt ± SD of indicated metal adsorbed (μg/cm²)[b]				
	Mn	Fe	Co	Ni	Cu
N1-C	0.15 ± 0.01	269.50 ± 29.90	0.05 ± 0.01	0.26 ± 0.04	0.09 ± 0.03
N1-B	0.17 ± 0.04	3,145.00 ± 198.06	0.05 ± 0.02	1.98 ± 0.01	0.81 ± 0.10
N2-C	0.06 ± 0.02	238.50 ± 29.05	0.07 ± 0.02	0.24 ± 0.09	0.23 ± 0.01
N2-B	20.09 ± 0.10	11,596.00 ± 238.45	0.25 ± 0.17	11.52 ± 0.36	2.18 ± 0.13
A1-C	0.06 ± 0.03	48.60 ± 9.30	0.01 ± 0.01	0.14 ± 0.03	0.01 ± 0.01
A1-B	0.18 ± 0.01	994.40 ± 137.09	NB[c]	0.07 ± 0.02	0.16 ± 0.04
A2-C	0.09 ± 0.02	9.55 ± 1.65	0.03 ± 0.01	0.18 ± 0.05	0.06 ± 0.01
A2-B	0.10 ± 0.02	50.00 ± 21.25	NB	0.69 ± 0.31	0.09 ± 0.02

[a] C, Control surface; B, biofilm.
[b] Figures represent an average of at least five determinations for each sample plus or minus the standard deviation of the mean. The biofilm values are expressed in terms of metal adsorbed in excess of the corresponding control surface.
[c] NB, Not bound.

are summarized in Table 1. Iron was the most abundant metal species at all sites, accounting for up to 83 mol% of the five assayed transition metals. Consecutively lower levels of nickel, manganese, cobalt, and copper were recorded, with a 3- to 100-fold concentration decrease for all metals in the circumneutral pH system. Of the two acidic sites, station A2 had the highest aqueous metal concentrations. This reflects the small size, upstream location, and limited dilution of Fraser Pond by casual runoff. In contrast, similar concentrations of manganese, cobalt, nickel, and copper were found at both neutral pH stations. However, approximately four times more iron was found at site N2. Since Lower Moose Lake tends to become partially meromictic during the summer months, site N2 was probably situated in or near an upwelling current of iron-rich anoxic water from the chemocline.

The biofilms bound each metal species, with the exception of cobalt under acidic conditions, in excess of the amounts adsorbed by the corresponding control surfaces (Table 2). Metal adsorption was enhanced at neutral pH values, particularly at site N2 where a thick (ca. 1.0-mm) coating of iron-oxide precipitates developed throughout the biofilms. Such ocherous deposits were not evident on the control strips and formed only thin biofilm-associated accretions at the other sample stations.

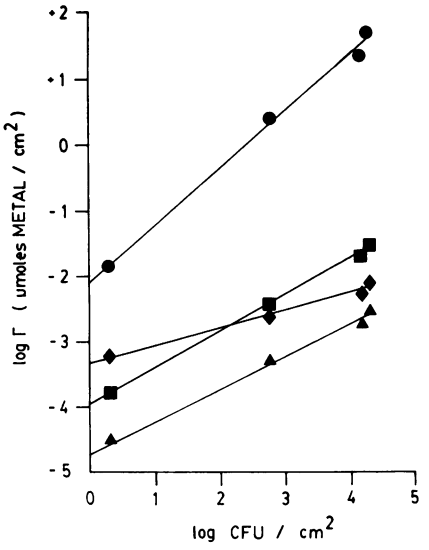

FIG. 3. Biofilm metal adsorption isotherms for Mn (▲), Fe (●), Ni (◆), and Cu (■) under neutral pH conditions at site N1.

A set of metal adsorption isotherms for the biofilms at site N1 are shown in Fig. 3. Similar plots were obtained with data from the other sample stations. In each case, regression analysis yielded straight lines with correlation coefficients of 0.98 to 0.99. These Freundlich-type isotherms provided estimates of the conditional metal-binding capacity constants (X_i) for the biofilms and an index of adsorption strength (θ_i) for each metal species (Table 3). The lower pX_i values at stations N1 and N2 show that biofilm metal adsorption was enhanced by up to 12 orders of magnitude over the two acidic sites. Also, the adsorption strength values associated with the metals were usually higher at elevated pH levels; iron was the only exception and exhibited similar θ_i values at all sites. These trends indicate that microbial biofilms are more efficient scavengers of metals under neutral pH conditions.

Biofilm mineralization. Microcolonies of encapsulated bacteria were commonly found in stained thin sections of the biofilms (Fig. 4). The extracellular polymeric material surrounding the cells typically exhibited a fibrous structure and often contained an abundance of iron-rich precipitates. In specimens from the two neutral pH stations, these deposits were distinguished by a granular appearance, whereas clumps of acicular crystalloids developed under acidic conditions (compare Fig. 4A and 4B). Both types of mineralization generated diffuse prismatic diffraction bands, with characteristic reflections for ferrihydrite ($Fe_2O_3 \cdot nH_2O$) centered on d equaling 0.25 and 0.15 nm. However, energy-dispersive X-ray spectroscopic analyses revealed distinct differences in trace element composition between the two

TABLE 3. Biofilm metal adsorption capacity constants and adsorption strength values

Metal	Values found in station:							
	N1		N2		A1		A2	
	θ_i	pX_i	θ_i	pX_i	θ_i	pX_i	θ_i	pX_i
Mn	2.04	4.71	1.47	3.21	0.25	17.08	0.32	13.85
Fe	1.16	2.09	1.08	1.63	1.06	2.39	1.28	3.23
Co	0.96	7.66	0.80	7.87	NB[a]	NB	NB	NB
Ni	1.82	3.98	1.41	4.08	0.63	8.34	0.75	6.67
Cu	2.24	3.31	2.08	3.58	0.42	11.38	0.58	9.09

[a] NB, Not bound.

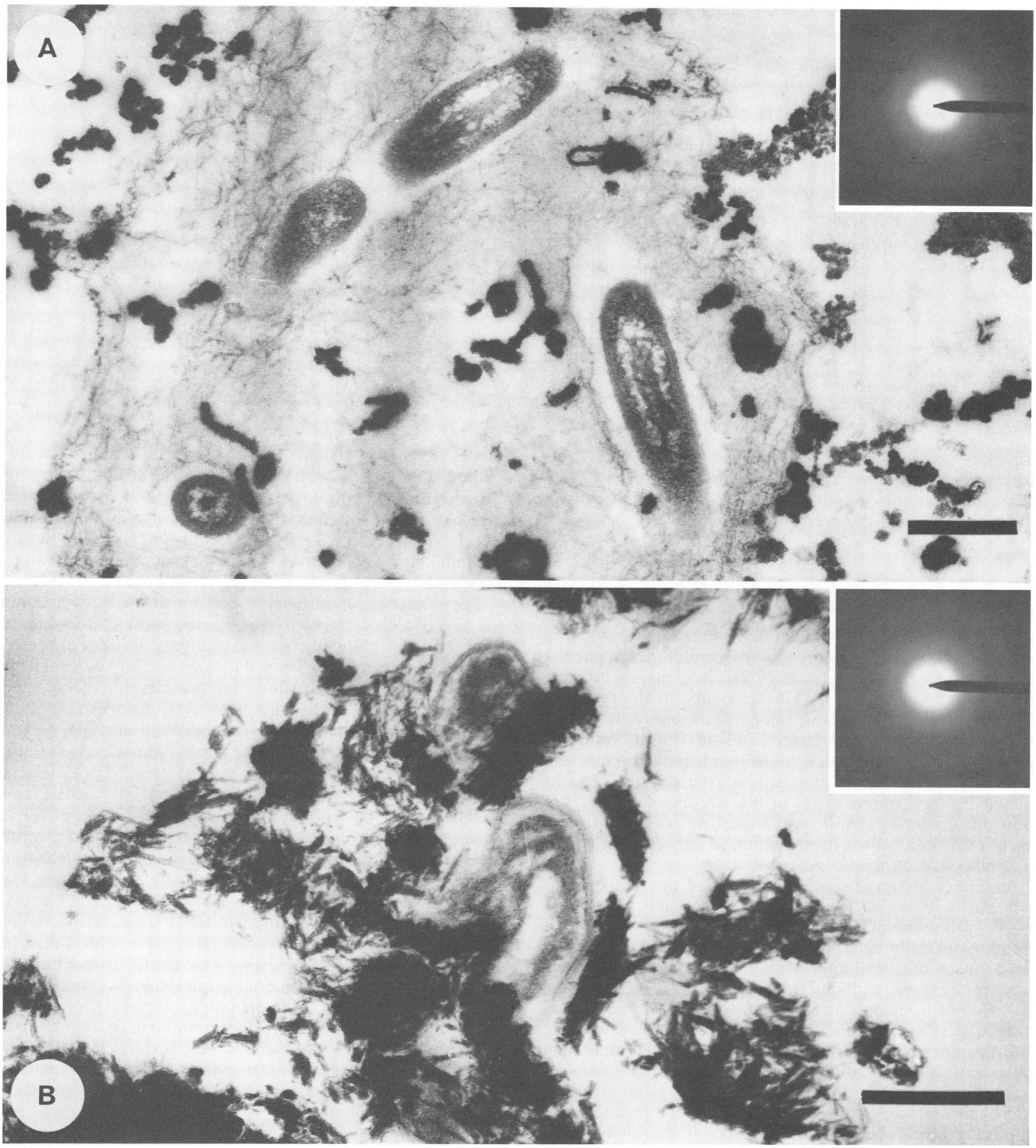

FIG. 4. Transmission electron micrographs of stained thin sections showing mineralized bacterial microcolonies in biofilms from sample stations N2 (A) and A1 (B). Insets show corresponding selected area electron diffraction patterns for the ferrihydrite precipitates (bars, 500 nm).

types of ferrihydrite precipitates (Fig. 5). Those which formed at neutral pH values contained Al, Si, and Cl as minor indigenous element impurities, whereas S was incorporated under acidic conditions.

The direct examination of unstained specimens provided electron-scattering profiles of microcolonies in early stages of mineralization. In these micrographs, individual cells typically appeared as ghosts enmeshed in an electron opaque matrix containing small crystallites (Fig. 6A). Energy-dispersive X-ray spectroscopy showed that iron was the principle source of contrast inherent to these specimens. Furthermore, a close association between the fibrous capsular

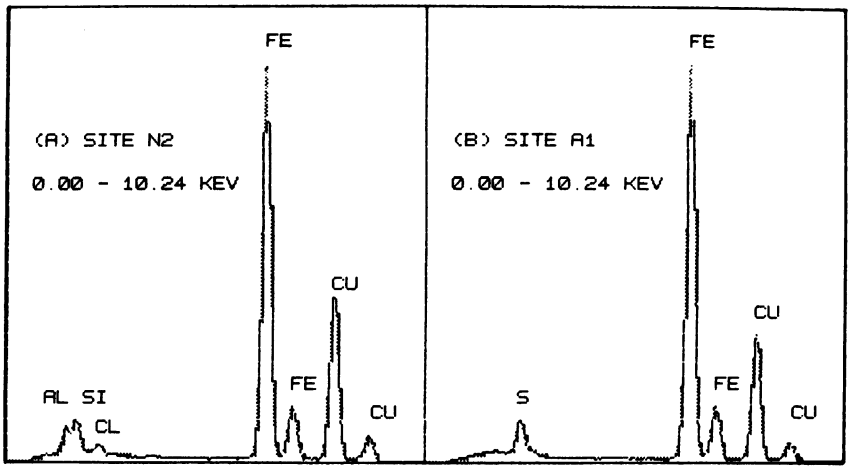

FIG. 5. Energy-dispersive X-ray spectra from ferrihydrite precipitates associated with biofilms at sites N2 (A) and A1 (B). Fe (Kα and Kβ), Al, Si, Cl and S peaks are from the specimens; Cu (Kα and Kβ) peaks are from the supporting grids.

polymers surrounding the bacteria and the nascent ferrihydrite precipitates could be discerned after staining (Fig. 6B). This confirmed that the attached bacteria served directly as nucleation sites for the precipitation of iron.

DISCUSSION

The plate counts for attached and planktonic heterotrophic bacteria were generally similar to those obtained in other aquatic environments stressed by metal-laden acid mine drainage (29, 40). Extremely low pH conditions, in particular, are known to adversely affect microbial metabolic activities and growth rates (34). This accounts for the relatively poor response to seasonal warming of suspended bacteria at the two immediately impacted sites in Fraser Pond and Cranberry Lake. Evidence of acid-induced stress is also seen in comparisons of the biofilm communities. The attached bacterial populations in the circumneutral pH systems demonstrated significantly higher rates of growth over the last 12 weeks of the investigation as expected for a more vigorous guild of microorganisms.

A number of recent studies suggest that the initial colonization of submerged surfaces of microorganisms is dominated by the simultaneous attachment and growth of individual planktonic cells (6, 27). This effectively supports a rapid onset of population growth, particularly when the concentration of free-living cells is high (cf. Fig. 2A through 2D). However, as the density of attached cells increases and surface area becomes limiting (i.e., fewer attachment sites), the attachment rate is greatly reduced (6). At the same time, emigration of cells from larger unstable microcolonies occurs with increasing frequency (27). The later stages of microbial surface colonization are therefore characterized by progressively slower rates of population growth. These observations explain the overall pattern of biofilm formation at each of our four sample stations.

The differences in biofilm metal adsorption between the neutral and acidic pH sites can be attributed partly to variations in population density. However, reactions between metallic ions and polymeric organic substrates are strongly influenced by pH level (39). This solution property determines the extent to which protons dissociate from reactive acidic groups and effectively controls surface charge density (24). At a low pH level, the availability of negatively charged sites such as carboxylates and phosphates is greatly reduced so fewer metal cations are adsorbed. Conversely, metallic ion adsorption is usually enhanced under neutral pH conditions by a proportional increase in the number of ionized acidic groups. These effects are reflected in the total amounts of metal bound by the biofilms (Table 2) and the corresponding pX_i values (Table 3).

The reduction of surface charge density with decreasing pH levels also serves to weaken electrostatic free energy contributions to metallic ion adsorption (24). This accounts for the observed trend towards lower (or unmeasurable in the case of cobalt) θ_i values for manganese, nickel, and copper at the two acidic sites. However, electrostatic interactions may additionally be weakened through the partial hydrolysis of a metal cation. The adsorption behavior of iron is explained by this later process. At elevated pH levels, cationic colloidal elements [i.e., $Fe(OH)^{2+} \cdot 5H_2O$ or $Fe(OH)^{+} \cdot 4H_2O$] tend to form via the hydrolysis of ferric iron (9). This probably caused a decrease in the overall strength of adsorption by counteracting the increase in electrostatic interactions which usually accompany the higher surface charge density expected at neutral pH levels.

Previous studies have demonstrated that bacterial cells are capable of serving as nucleation sites for a wide range of authigenic minerals (4, 13, 14). The ability of bacteria to serve as templates for mineralization depends primarily on the inherent capacity of their anionic surface polymers to bind metallic ions (3, 12). Once immobilized, complexed metals can be precipited at the cell surface by complete hydrolysis, a change in oxidation state, or through reactions with other counter ions in solution (14). The secondary growth of these mineral precipitates may then proceed via homogeneous crystal nucleation reactions (2). A similar series of events starting with the oxidation and hydrolysis of cell-bound ferrous or ferric iron probably accounts for the mineralization which developed in association with the biofilms.

The morphological characteristics of the ferrihydrite mineralization associated with the biofilms correlates well with the trace element composition of the precipitates. Synthesis experiments have shown that the incorporation of silicate or sulfate anions not only suppresses the ordering of ferrihydrite but also hinders the formation of more stable anhydrous iron oxides such as goethite or hematite (1, 5, 8). The

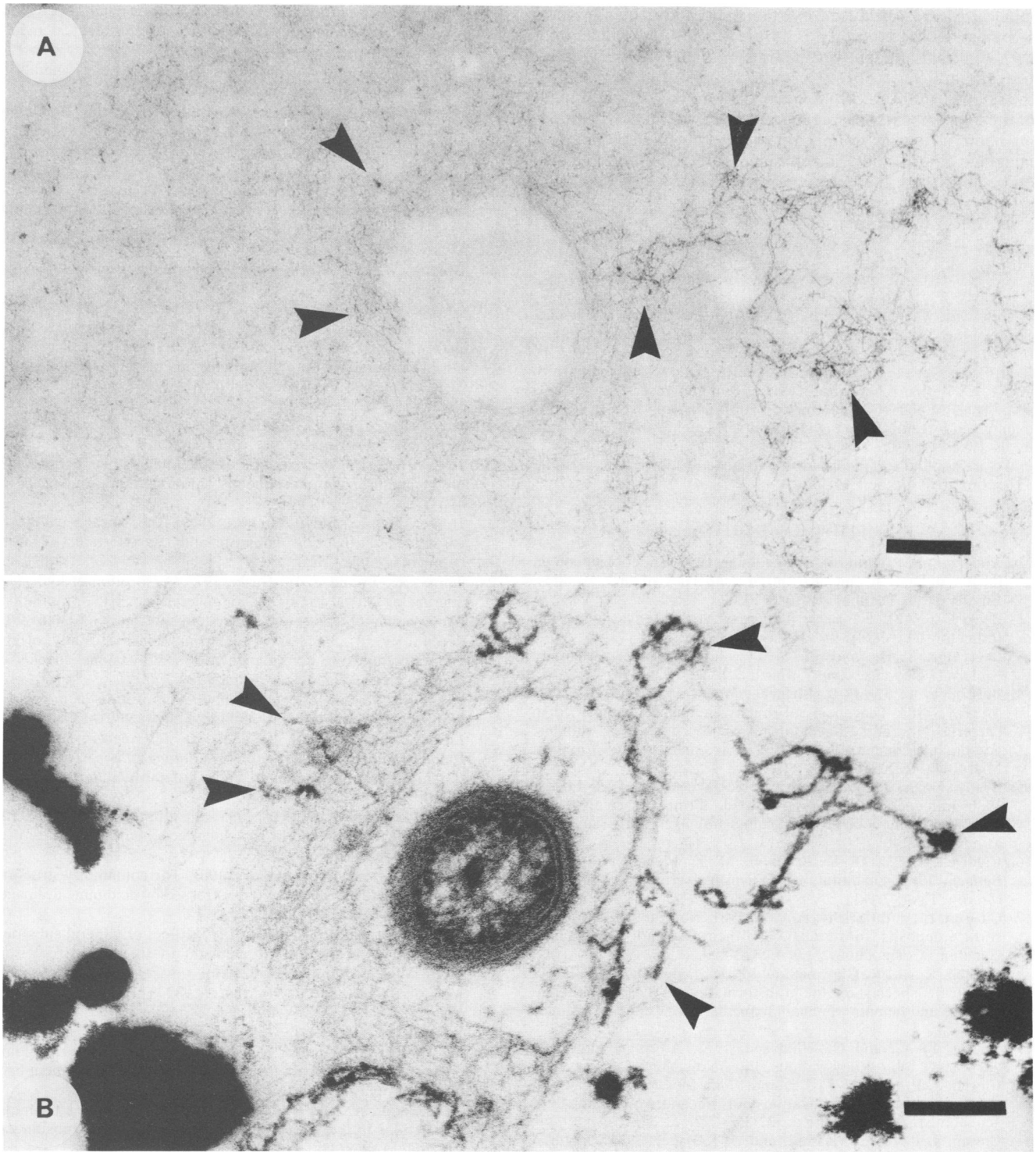

FIG. 6. Thin-section profiles of encapsulated bacteria in unstained (A) and stained (B) biofilm specimens from site N2. Arrows indicate some nascent ferrihydrite precipitates associated with the extracellular polymers of the bacteria (bars, 200 nm).

exact reasons for this are not yet clear. However, the available evidence suggests that negatively charged ions effectively cross-link colloidal particles of ferrihydrite and promote the formation of an unreactive immobile mineral phase (8). In the case of sulfate, this seems to result in the production of fibrous networks of acicular ferrihydrite crystalloids similar to those observed in association with biofilms from the acidic sites (5). However, siliceous ferrihydrites commonly exhibit a more granular morphology reminiscent of the iron precipitates which developed in conjunction with the neutral pH biofilms (7, 8).

This work heightens our awareness of the importance of microbial biofilms as natural metal-immobilizing matrices. Clearly, there are differences between metal affinities and

mineral species produced by the acidic and neutral natural systems reported here; this is presumably a reflection of their different aqueous chemistries and microbial communities. In both cases, metal sorption went well beyond that of the control surfaces. Preliminary data on the the microbiota of the deep subsurface (21) suggest that mineral development on bacterial surfaces may be more widespread than first imagined (Fig. 1 and 2 in reference 21). Given the antiquity of bacteria and the terrestrial depths at which they can reside, microbial metal immobilization and mineralization cannot be a trivial affair (3).

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LITERATURE CITED

1. **Anderson, P. R., and M. M. Benjamin.** 1985. Effect of silicon on the crystalization and adsorption properties of ferric oxides. Environ. Sci. Technol. **19**:1048–1053.
2. **Berner, R. A.** 1980. Early diagenesis: a theoretical approach. Princeton University Press, Lawrenceville, N.J.
3. **Beveridge, T. J., and W. S. Fyfe.** 1985. Metal fixation by bacterial cell walls. Can. J. Earth Sci. **22**:1892–1898.
4. **Beveridge, T. J., J. D. Meloche, W. S. Fyfe, and R. G. E. Murray.** 1983. Diagenesis of metals chemically complexed to bacteria: laboratory formation of metal phosphates, sulfides, and organic condensates in artificial sediments. Appl. Environ. Microbiol. **45**:1094–1108.
5. **Brady, K. S., J. M. Bigham, W. F. Jaynes, and T. J. Logan.** 1986. Influence of sulfate on Fe-oxide formation: comparisons with a stream receiving acid mine drainage. Clays and Clay Miner. **34**:266–274.
6. **Caldwell, D. E., D. K. Brannan, M. E. Morris, and M. R. Betlach.** 1981. Quantitation of microbial growth on surfaces. Microb. Ecol. **7**:1–11.
7. **Carlson, L., and U. Schwertmann.** 1981. Natural ferrihydrites in surface deposits from Finland and their association with silica. Geochim. Cosmochim. Acta **45**:421–429.
8. **Cornell, R. M., R. Giovanoli, and P. W. Schnidler.** 1987. Effect of silicate species on the transformation of ferrihydrite into goethite and hematite in alkaline media. Clays and Clay Miner. **35**:21–28.
9. **Cotton, F. A., and G. Wilkinson.** 1972. Advanced inorganic chemistry. John Wiley & Sons, Inc., New York.
10. **Cowen, J. P., and K. W. Bruland.** 1985. Metal deposits associated with bacteria: implications for Fe and Mn marine biogeochemistry. Deep-Sea Res. **32**:253–272.
11. **Cowen, J. P., G. J. Massoth, and E. T. Baker.** 1986. Bacterial scavenging of Mn and Fe in a mid- to far-field hydrothermal particle plume. Nature (London) **322**:169–171.
12. **Degens, E. T., and V. I. Ittekkot.** 1981. *In situ* metal staining of biological membranes in sediments. Nature (London) **298**:262–264.
13. **Ferris, F. G., T. J. Beveridge, and W. S. Fyfe.** 1986. Iron-silica crystallite nucleation by bacteria in a geothermal sediment. Nature (London) **320**:609–611.
14. **Ferris, F. G., W. S. Fyfe, and T. J. Beveridge.** 1987. Bacteria as nucleation sites for authigenic minerals in a metal contaminated lake sediment. Chem. Geol. **63**:225–232.
15. **Fletcher, M.** 1985. Effect of solid surfaces on the activity of attached bacteria, p. 339–362. *In* D. C. Savage and M. Fletcher (ed.), Bacterial adhesion: mechanisms & physiological significance. Plenum Publishing Corp., New York.
16. **Forstner, U.** 1982. Accumulative phases for heavy metals in limnic sediments. Hydrobiologia **91**:269–284.
17. **Geesey, G. G., R. Mutch, J. W. Costerton, and R. B. Green.** 1978. Sessile bacteria: an important component of the microbial population in small mountain streams. Limnol. Oceanogr. **23**: 1214–1223.
18. **Geesey, G. G., W. T. Richardson, H. G. Yeomans, R. T. Irvin, and J. W. Costerton.** 1977. Microscopic examination of natural sessile bacterial populations from an alpine stream. Can. J. Microbiol. **23**:1733–1736.
19. **Ghiorse, W. C.** 1984. Biology of iron and manganese depositing bacteria. Annu. Rev. Microbiol. **38**:515–550.
20. **Ghiorse, W. C.** 1986. Applicability of ferromanganese—depositing microogranisms to industrial metal recovery processes, p. 141–148. *In* H. L. Erlich and D. S. Holmes (ed.), Workshop on biotechnology for the mining, metal-refining and fossil fuel processing industries. John Wiley & Sons, Inc., New York.
21. **Ghiorse, W. C., and J. T. Wilson.** 1988. Microbial ecology of the terrestrial subsurface. Adv. Appl. Microbiol. **33**:107–172.
22. **Huljev, D. J.** 1986. Interaction of some metals between marine-origin humic acids and aqueous solutions. Environ. Res. **40**: 470–478.
23. **Hunter, K. A., and P. S. Liss.** 1979. The surface charge of suspended particles in estuarine and coastal waters. Nature (London) **282**:823–825.
24. **James, R. O., and T. W. Healy.** 1972. Adsorption of hydrolyzable metal ions at the oxide-water interface. III. A thermodynamic model of adsorption. J. Colloid Interface Sci. **40**:65–81.
25. **Jardim, W. F., and H. W. Pearson.** 1984. A study of the copper-complexing compounds released by some species of cyanobacteria. Water Res. **18**:985–989.
26. **Kerndorf, H., and M. Schnitzer.** 1980. Sorption of metals on humic acid. Geochim. Cosmochim. Acta **44**:1701–1708.
27. **Lawrence, J. R., P. J. Delaquis, D. R. Korber, and D. E. Caldwell.** 1987. Behavior of *Pseudomonas fluorescens* within the hydrodynamic boundry layers of surface microenvironments. Microb. Ecol. **14**:1–14.
28. **Mann, H., and W. S. Fyfe.** 1985. Algal uptake of U and some other metals: implications for global geochemical cycling. Precambrian Res. **30**:337–349.
29. **Mills, A. L., and L. M. Mallory.** 1987. The community structure of sessile heterotrophic bacteria stressed by acid mine drainage. Microb. Ecol. **14**:219–232.
30. **Mills, A. L., and R. Maubrey.** 1981. Effect of mineral substrate composition on bacterial attachment to submerged rock surfaces. Microb. Ecol. **7**:315–322.
31. **Millward, G. E., and R. M. Moore.** 1982. The adsorption of Cu, Mn and Zn by iron oxyhydrate in model estuarine solution. Water Res. **16**:981–985.
32. **Mittelman, M. W., and G. G. Geesey.** 1985. Copper-binding characteristics of exopolymers from a freshwater-sediment bacterium. Appl. Environ. Microbiol. **49**:846–851.
33. **Nissenbaum, A., and D. J. Swaine.** 1976. Organic matter-metal interactions in recent sediments: the role of humic substances. Geochim. Cosmochim. Acta **40**:809–816.
34. **Palumbo, A. V., M. A. Boyle, R. R. Turner, J. W. Elwood, and P. J. Mulholland.** 1987. Bacterial communities in acidic and circumneutral streams. Appl. Environ. Microbiol. **53**:337–344.
35. **Reid, J. D., and B. McDuffie.** 1981. Sorption of trace cadmium on clay minerals and river sediments: effects of pH and Cd (II) concentrations in a synthetic river water medium. Water Air Soil Pollut. **15**:375–386.
36. **Reuter, J. H., and E. M. Perdue.** 1977. The importance of heavy metal-organic interactions in natural water. Geochim. Cosmochim. Acta **41**:325–334.
37. **Rudd, T., R. M. Sterritt, and J. N. Lester.** 1984. Formation and

conditional stability constants of complexes formed between heavy metals and bacterial extracellular polymers. Water Res. **18**:379–384.
38. **Schoer, J.** 1985. Iron-oxo-hydroxides and their significance to the behavior of heavy metals in esturaries. Environ. Technol. Lett. **6**:189–202.
39. **Stumm, W., and J. J. Morgan.** 1981. Aquatic chemistry. John Wiley & Sons, Inc., New York.
40. **Wassel, R. A., and A. L. Mills.** 1983. Changes in water and sediment bacterial community structure in a lake receiving acid mine drainage. Microb. Ecol. **9**:155–169.
41. **Weise, W., and G. Rheinheimer.** 1978. Scanning electron microscopy and epifluorescence investigation of bacterial colonization of marine sand sediments. Microb. Ecol. **4**:175–188.