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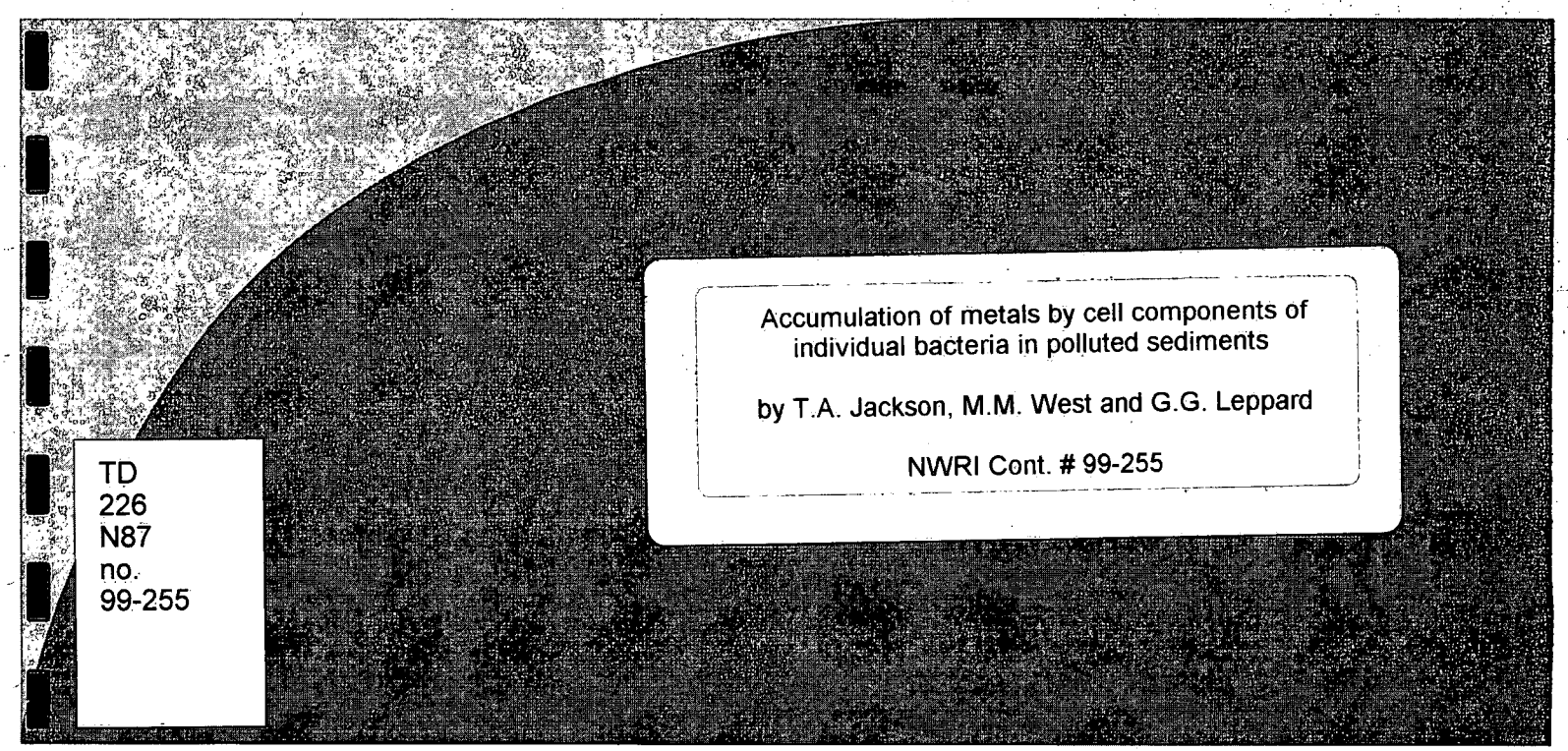

Accumulation of metals by cell components of individual bacteria in polluted sediments

by T.A. Jackson, M.M. West and G.G. Leppard

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Energy dispersive X-ray microanalysis combined with transmission electron microscopy is a unique, powerful, and refined technique for determining the composition of individual microscopic components of heterogeneous natural materials. This method permits regions less than one micrometre in diameter within a single bacterial cell or microscopic particle to be selected visually and then analysed rapidly and semi-quantitatively for a wide spectrum of metallic and nonmetallic elements. Thus, it yields valuable information that would be impossible to obtain by crude conventional methods such as bulk analysis. Using energy dispersive X-ray microanalysis and transmission electron microscopy, we investigated the preferential accumulation and partitioning of copper (Cu) and other elements by cell components of bacteria (cell walls, cytoplasm, and cytoplasmic inclusions) and associated nonliving material (mineral coatings on cells and fibrils) in a sample of sediment from Larder Lake, a Northern Ontario lake polluted with mine tailings. By amassing statistically meaningful quantities of data, we were able to assess the associations of different elements with each other and with various biological and inorganic micro-structures; this, in turn, provided a basis for interpreting our results in terms of biogeochemical processes.

Our results are consistent with the following interpretations: (1) Bacterial cells and fibrils in the sediment are generally coated with iron and manganese oxyhydroxides (FeOOH and MnOOH) deposited *in situ*. (2) Cu is strongly bound to these mineral coatings and is not readily displaced from the binding sites by competing elements such as Ca, Mg, and K. (3) Cu is also bound directly by ligands belonging to cell wall biopolymers. (4) The cell wall sorption sites are more abundant than the oxyhydroxide sorption sites, but they bind Cu more weakly; consequently, Cu is readily displaced from the cell wall sites by Ca, Mg, and K. (5) Deposits of Si-, Al-, and Fe-bearing mineral deposits (clay minerals?) on the cell walls tend to block the uptake of Cu by cell wall binding sites. (6) Cu is preferentially accumulated either by the oxyhydroxide coatings or by Fe-, Mn-, and P-rich inclusions in the cytoplasm, depending on the nature of the individual bacterium; this suggests that different kinds of bacteria employ a number of very different detoxification mechanisms, and prevention of metal uptake by the cell is only one of them (and probably not the most important one). Possibly our results could lead to applications in the field of pollution control technology.

Finally, I could add that our paper represents significant pioneer work in an important but poorly known and largely neglected field of environmental geochemistry. In our view, we have made a fundamental innovative contribution which is likely to arouse wide interest among scientists, technologists, and research managers in a number of disciplines concerned with environmental problems (biogeochemistry, microbial ecology, pollution control technology, and others), thereby stimulating further advancement of knowledge and useful technology on different fronts. Through our novel application of highly sophisticated modern techniques that are rarely used in work of this nature, we have opened up a new dimension of environmental research. In the process, we have contributed valuable new information and insights pertaining to ecologically significant biogeochemical processes on a microscopic scale in sediments. More specifically, please note the following points which set our work apart from more conventional research on heavy metals: (1) We analysed specific components of individual bacterial cells and associated nonliving material. (2) Our specimens came from a sample of natural sediment rather than a laboratory culture; hence, they represent microbes and particles in their native state and natural associations of microbes, nonliving particles, microenvironments, and specific metals and nonmetallic elements as they actually exist at a contaminated site in a polluted lake. (3) Our analyses produced quantitative data for statistically meaningful numbers of specimens, permitting us to assess relationships of elements with each other and with specific biological and inorganic microstructures using rigorous statistical techniques. (4) The samples were preserved in the field immediately after being collected, eliminating the problem of deterioration during storage. (5) To the best of our knowledge, we are the first investigators in this field to classify metal-accumulating coatings on bacterial cells in nature. (6) We found evidence for different mechanisms whereby microbes accumulate (and possibly detoxify) heavy metals such as Cu. It is noteworthy that accumulation inside the cell is at least as important as accumulation in cell wall coatings.

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Accumulation of Heavy Metals by Individually Analyzed Bacterial Cells and Associated Nonliving Material in Polluted Lake Sediments

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The microfloras of sediments control the biogeochemical cycling of heavy metals in natural waters, thereby determining, in large part, the ecological effects of the metals. However, little is known about interactions of metals with individual benthic microbes in nature owing to the technical difficulty of studying such small-scale processes in complex, heterogeneous sedimentary ecosystems. Seeking to overcome this obstacle, we used energy-dispersive X-ray microanalysis and transmission electron microscopy to investigate the accumulation of Cu and other elements by the cell walls, cytoplasm, cytoplasmic inclusions, fibrils, and mineral coatings of individual bacterial cells in the sediments of a lake polluted with heavy metals. Data from statistically meaningful numbers of specimens revealed various associations of elements with each other and with specific microstructures. The results imply (i) common occurrence of Fe and Mn oxyhydroxide coatings on cell walls and fibrils; (ii) strong sorption of Cu by these coatings and the inability of Ca, Mg, and K to displace the Cu from the binding sites; (iii) weaker but more extensive direct sorption of Cu by cell walls from which the Cu is readily displaced by Ca, Mg, and K; (iv) blockage of cell wall ligands by Si-, Al-, and Fe-bearing mineral deposits; and (v) preferential accumulation of Cu in the coatings or in cytoplasmic inclusions enriched in Fe and usually enriched in P as well (with or without measurable enrichment in Mn) with respect to the cytoplasm. Our findings help to elucidate the accumulation of heavy metals by microbes in aquatic ecosystems and suggest the existence of diverse mechanisms of detoxification.

Introduction

Fine-grained sediments and associated benthic communities in natural waters make up an assortment of ecosystems characterized by enormous complexity, heterogeneity, and variability on a microscopic scale (*1*). They are vitally important to the aquatic biota as a whole, because they act as both sinks and secondary sources of nutrients and toxic contaminants and mediate chemical transformations of these substances, thereby controlling their bioavailability. Thus, heavy metals are efficiently bound and accumulated by these sediments but are also subject to partial release into the overlying water. The binding, release, and speciation of metals in sediments involve many different processes (including sorption, desorption, complexing, oxidation–reduction reactions, and bioaccumulation) that are dependent on a wide range of spatially and temporally varying environmental and biological factors (such as pH, E_h, dissolved O_2, organic and inorganic ligands, cations of other metals, and microbial activities) (*2*). These processes are, to a great extent, mediated directly or indirectly by bacteria, which, together with nonliving metal-binding agents, such as colloidal iron and manganese oxyhydroxides (FeOOH and MnOOH), FeS, and humic matter, tend to dominate the biogeochemical cycles of the metals (*1–4*).

In-depth investigation of such phenomena under natural conditions has been hampered, however, by technical limitations. Much of the published information about sediment chemistry was generated by relatively crude methods such as analysis of bulk samples and sample fractions, and the study of benthic microfloras in their native state is still in its infancy. Thus, our understanding of sediment chemistry and microbiology is largely confined to gross properties of the sediments, to gross effects (which are actually the *net* effects of many events and processes occurring on a microscopic scale), and to characteristics of certain isolated components and inhabitants of the sediments. Our knowledge of the nature and in situ activities of bacteria in sediments and of interactions of the bacteria with each other and with associated nonliving matter is far too limited considering the crucial geochemical and ecological functions of these organisms (*5*). Chemists and microbiologists are striving to make good this deficiency (*6–8*); but there is little direct, detailed knowledge of relations between heavy metals and the myriads of diverse microbes and particles intermingled in sediments (let alone subunits of those entities) under natural conditions.

Energy-dispersive X-ray microanalysis (EDXM) combined with transmission electron microscopy (TEM) can, however, provide information about the in situ distribution of heavy metals and other elements among the various particles and microbes in sediments, as it has the unique advantage of allowing microscopic components of heterogeneous natural materials and even subunits of these entities (e.g., inclusions in bacterial cells) to be selected visually and then analyzed *individually* for a wide range of metallic and nonmetallic elements (*9–15*). The microscope's electron beam is focused on a chosen minute area of the specimen, and the abundances (though usually not the absolute concentrations) of all detectable elements in the irradiated region are determined rapidly by measuring characteristic X-rays emitted by their atoms (*9*).

The value of the EDXM method for environmental research has been greatly enhanced by several technical refinements (*12, 14, 15*) that have come into general use since the late 1980s: (i) the use of "ultrathin" (~0.05–0.1 μm thick) sample sections (prepared by embedding specially preserved specimens in epoxy resin or melamine and slicing them with an ultramicrotome, preferably employing a diamond knife) to minimize beam spreading and emission of background radiation within the target and to maximize

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TABLE 1. Selected Background Data for Water and Sediments at the Larder Lake Sampling Site[a]

sample	parameter	value ($\bar{X}$ or md and range, or single value)
water 1 m below surface	dissolved O_2 concn (mg/L)	8.75
	pH	7.60
	temp (°C)	18.8
	Cu concn (μg/L)	14
water 1 m above bottom	dissolved O_2 concn (mg/L)	9.8
	pH	7.16
	temp (°C)	5.8
	Ca concn (mg/L)	13.13
	Mg concn (mg/L)	4.27
	K concn (mg/L)	0.78
	Cu concn (μg/L)	16
surface sediment (to a depth of 4 cm)	pH	6.80 (6.70–6.90)
	E_h	+310
	clay concn (%)	62.99
	silt concn (%)	36.27
	sand concn (%)	0.74
	organic C concn (mg/g)	12.05 (10.7–13.4)
	sulfide concn (μg/g)	4.97 (4.94–5.00)
	$NH_2OH{\cdot}HCl/HNO_3$-extractable Mn concn (mg/g)	2.154 (2.083–2.225)
	$NH_2OH{\cdot}HCl/HNO_3$-extractable Fe concn (mg/g)	3.012 (2.780–3.244)
	Mn/Fe mol ratio of the $NH_2OH{\cdot}HCl/HNO_3$ extract	0.7295 (0.6975–0.7616)
	citrate/dithionite-extractable Fe concn (mg/g)	17.41 (16.65–18.17)
	total Cu concn (μg/g)	912 (891–934)
	Cu concn in pore water (mg/L)	2.01 (1.63–2.39)

[a] Sediment composition is expressed on the basis of dry weight. Abbreviations: $\bar{X}$ = mean; md = median; concn = concentration. Sources of data: Mudroch et al. (*35*) and T. A. Jackson (unpublished data).

visual definition of ultrastructure; (ii) a spatial resolution of ~0.001–0.003 μm for microscopic examination; (iii) short irradiation times (a few tens of seconds); and (iv) irradiation of extremely small (submicrometer) regions of the specimens. Microanalysis of bacteria in cultures and in the few field samples that have been examined has shown that their cell walls can scavenge heavy metals from ambient solutions (*16*). Functioning in some cases as templates, cell walls along with cell capsules and extracellular fibrils may also nucleate epitaxial precipitation of colloidal minerals, including FeOOH, MnOOH, and Fe–Al silicates, as coatings (*11–13, 15–20*). Furthermore, porous capsules and aggregates of extracellular fibrils may entrap mineral particles through the aggregation mechanisms suggested by Buffle et al. (*21*). These mineral deposits may enhance the microbial accumulation of heavy metals by sorbing or coprecipitating the metals, retaining them more tenaciously than cell wall polymers alone (*18*). Preferential sorption of specific metals is possible as well (*22–24*). The limited available evidence suggests that such phenomena are common among bacteria (*24–29*) and could therefore play significant roles in the biogeochemistry of heavy metals. The binding of metals by FeOOH and MnOOH is an important, widespread phenomenon in nature (*6, 30, 31*), and bacteria are abundant and nearly ubiquitous. However, the application of EDXM and TEM to processes of this kind in *natural* environments is still at a preliminary stage. Research along these lines has been largely confined to experimentally manipulated microbial cultures, whereas few natural assemblages have been examined. In research on natural materials, moreover, the most advanced techniques have been used only in a few recent studies. Besides, such work has been essentially qualitative as it has not involved the collection, quantification, and statistical analysis of data from multiple specimens. [A few quantitative studies have been performed (*23, 32, 33*), but they involved scanning electron microscopy rather than TEM.]

Field Site, Materials, Methods, and Background Data. In our research, modern EDXM and TEM techniques (*14, 15*) were applied to statistically meaningful numbers of bacteria and nonliving particles in lake mud ("statistically meaningful" being defined as sufficient to reveal correlations between covariant elements at a significance probability of ≤0.05 in most cases). Our purpose was to examine and quantify (i) the partitioning of heavy metals among different visually recognizable classes of microstructures and (ii) the relations between different elements, amassing enough data to enable us to assess the variations with the aid of statistics and to draw inferences about biogeochemical processes.

Grab samples of clay-silt sediment were collected from the main basin of Larder Lake, a small, well-oxygenated circumneutral Boreal forest lake on the Precambrian Shield of Northern Ontario, Canada. This lake was chosen for study because it is polluted with heavy metals from gold mine tailings (*34, 35*). The depth of water at the sampling site was 29.5 m, and the samples were taken in August when the water column was thermally stratified. A selection of data representing gross characteristics of the water and sediments at the field site is given in Table 1. The water near the bottom was rich in dissolved O_2 despite stratification of the water column (Table 1). Accordingly, the sediments had a high oxidation–reduction potential (E_h) and appreciable concentrations of operationally defined ($NH_2OH{\cdot}HCl/HNO_3$ and citrate/dithionite-extractable) FeOOH and MnOOH (Table 1). Also note that laboratory assays were performed on sediment samples to measure general microbial activity as represented by CO_2 production. Sealed flasks containing sediment–water slurries under an atmosphere of air or the inert gas helium were incubated in the dark for different lengths of time up to a maximum of 10–14 days, and the CO_2 content of the headspace was determined. The results showed a progressive increase in CO_2 level over time, indicating the presence of a viable microbial community (T. A. Jackson, unpublished data).

Specimens of surface sediment to be analyzed by the EDXM technique were preserved by immersion in 2% glutaraldehyde + 0.1 M sodium cacodylate buffer (pH 7.0); this was done in the field immediately after sample collection. The samples were then fixed in 1% OsO_4 in cacodylate

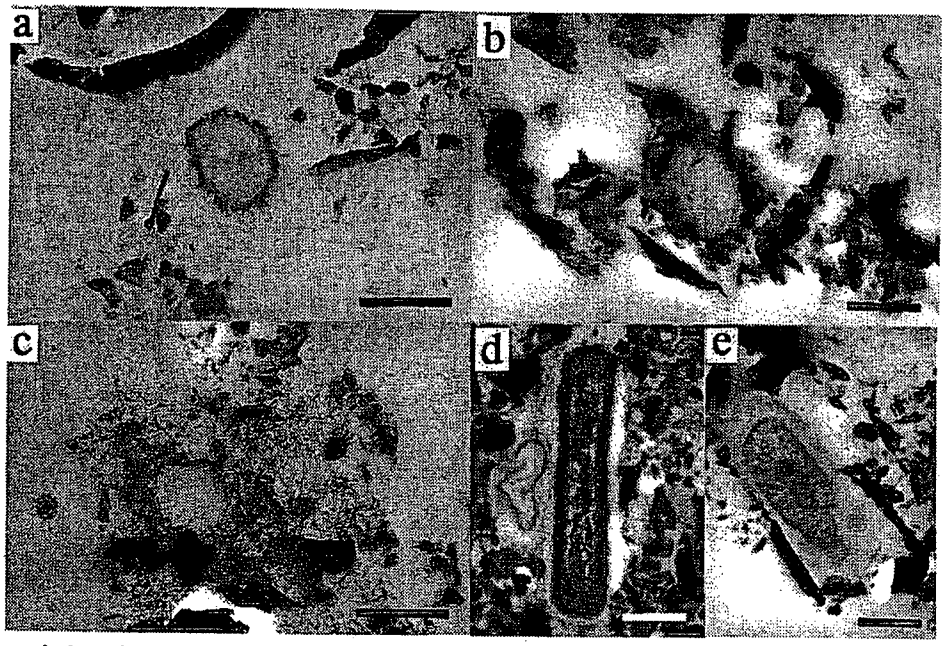

FIGURE 1. Transmission electron micrographs of representative coated and uncoated bacteria in Larder Lake sediment (not counterstained): (a) cell with coarse Fe-rich coating (probably FeOOH) devoid of detectable Mn, Si, or Al; (b) cell with coarse Si-rich, Al- and Fe-bearing, but Mn-free coating (possibly an aggregate of clay and FeOOH); (c) cell surrounded by fibrils and a fine Fe-rich coating (probably FeOOH) with some Si and Al (possibly clay) but no Mn; (d) uncoated cell; (e) uncoated cell containing a cytoplasmic inclusion (probably polyphosphate with sorbed or coprecipitated Fe species) that is enriched in Fe and, above all, P with respect to the cytoplasm. Length of scale bar: 0.5 μm.

solution, subjected to stepwise dehydration by treatments with ethanol, and impregnated with Spurr's resin in ethanol. Following stepwise removal of the ethanol, the resin was polymerized by heating at 70 °C, and ultrathin (~0.08–0.11 μm) sections were cut with an RMC MT 7 ultramicrotome employing a diamond knife. The sections were laid out on Formvar-coated nylon grids and examined by TEM using a JEOL JEM 1200 EX-II TEMSCAN electron microscope operating at 80 kV, and selected sediment constituents were analyzed with a Princeton Gamma Tech PGT IMIX-II microanalysis system using the same values of instrument parameters for all analyses and expressing all data as counts/240 s. The apparatus and analytical techniques that we employed are suitable for any element of atomic number >10. The minimum detectable mass fraction of a given element is a function of the atomic number and specific properties of the element, ranging from 0.1% to 0.5% for elements of biogeochemical interest. Because our analyses represented the initial stage in a longer term investigation of sediments using EDXM and TEM techniques and were performed on complex, heterogeneous natural materials for which the suitability of specific standard substances has not been established, we did not attempt (nor did we need) to achieve absolute quantification; instead, we determined the abundances of all elements present in measurable amounts, and then examined element associations, in minute, precisely pinpointed regions of selected microscopic components of the sediment. The selected sediment constituents were chosen on the basis of recognizable morphology and thus covered a considerable range of sizes, extending from less than to greater than section thickness. Because our goal was to discover and quantify element associations and to estimate their significance by statistical analysis, not to determine absolute concentrations of elements, it was not necessary to attempt to correct the total counts per analysis for effects of variations in section thickness. More rigorous analytical techniques, such as the use of local peak-to-background ratios, may be adopted at a more advanced stage of our research.

Results and Discussion

Electron microscopic examination of sediment sections revealed many scattered bacterial cells mingled with minute mineral grains and organic particles (Figure 1). Some cells contained intact cytoplasm in which electron-dense inclusions were occasionally found, whereas others were empty, and certain cells were surrounded by fibrils. Out of 71 cells analyzed by EDXM, 59 (83%) were evidently coated with mineral deposits. The cell walls, coatings, fibrils, and cell contents had variable quantities of Fe, Mn, Si, Al, Ca, Mg, K, P, Cu, and Zn. Although detectable levels of all of these elements did not occur in all specimens, the presence of measurable quantities of Fe was universal, and Cu was the most common and abundant heavy metal from the mine tailings. Cell walls were defined as "coated" on the basis of two criteria: (i) the presence of electron-dense material ranging from coarse mineral particles adsorbed to the cell wall or penetrating it, forming an evenly distributed or patchy armour-like coating (Figure 1a,b), to fine, diffuse impregnations in cell walls and fibrils (Figure 1c); and (ii) generally high concentrations of Fe with or without Mn and with or without Si and Al in presumptive coatings and fibrils relative to uncoated cell walls and cytoplasm (Table 2). Even "uncoated" cell walls (Figure 1d,e) had detectable Fe (Table 2), suggesting the formation of incipient coatings. Fibrils were usually enriched in Fe and commonly enriched in Mn as well. Most of the Fe and Mn associated with cell walls and fibrils probably represented FeOOH and MnOOH precipitated in situ; such deposits commonly occur on exposed surfaces in well-oxygenated aquatic environments and are actively or passively precipitated by bacteria on the surfaces of their cells and fibrils (*25*, *36*). However, the Si and Al (and some of the Fe) may represent clay minerals (adsorbed detrital clay or "authigenic" clay formed in situ). Among coatings containing Si and Al, the coarser ones were, on average, richer in those elements than the finer ones (not shown). The coexistence of bacteria characterized by coatings that differ so greatly in appearance and composition suggests a wide

TABLE 2. Fe, Mn, and Cu Content of Cell Coatings, Uncoated Cell Walls, and Cytoplasm of Bacteria[a]

description of specimens	Fe				Mn				Cu							
									alkali & alkaline earth metals present				alkali & alkaline earth metals absent			
	$\bar{X}$	SD	range	N	$\bar{X}$	SD	range	N	$\bar{X}$	SD	range	N	$\bar{X}$	SD	range	N
							Coatings									
coarse, even	3983	4076	352–13142	12	12615	8,371	3002–18297	2	535	363	244–1266	8	663	262	450–983	3
coarse, patchy	2423	1495	663–7421	23	469	256	154–793	4	317	104	121–477	13	733	239	400–1024	6
fine, fibrillar	3435	2096	653–8106	17	1505	1446	295–3736	8	331	154	144–542	9	933.5	1038	162–2830	7
fine, nonfibrillar	4,421	5854	536–18298	8	5,763	7,767	350–17096	4	704	773	238–1858	4	445			1
							Uncoated Cell Walls									
	742	89.7	655–860	4	nd			4	269	86.9	202–367	3	286			1
							Cytoplasm of Coated Cells									
coarse, even coating	1438	1675	298–3883	4	983			1	442	29.7	421–463	2	640.5	384	369–912	2
coarse, patchy coating	670.5	135	575–766	2	nd			2	294			1	394			1
fine, fibrillar coating	501	277	305–697	2	nd			2	361			1	777			1
fine, nonfibrillar coating				0				0				0				0
							Cytoplasm of Uncoated Cells									
	1016	685	285–1935	7	666			1	587	279	336–873	4	475	305	281–826	3

[a] Concentration units: counts/240 s. Symbols: $\bar{X}$ = mean; SD = standard deviation; N = number of cells represented by the data; nd = not detected.

FIGURE 2. Variation of Cu content with respect to Mn (a) and Fe (b) content in coatings of bacterial cells in Larder Lake sediment. Data for interiors of empty coated cells were included. Solid symbols: at least one of the elements Ca, Mg, and K is present; open symbols: detectable Ca, Mg, and K are absent. Types of coating represented: coarse, even (●, ○); coarse, patchy (■, □); fine, fibrillar (▲, △); fine, nonfibrillar (▼, ▽). The regression lines, correlation coefficients (r values), and significance probabilities (P values) pertain solely to the points marked by solid symbols.

variety of microenvironments surrounding the cells in accordance with theories put forward by microbial physiologists and colloid chemists (*37, 38*). The evenly distributed coatings were probably formed in situ, but mineral particles attached to cells in a patchy, random fashion could have been sorbed fortuitously.

Cu was detected in many samples of bacterial cell coatings (Table 2). Among coatings containing measurable Cu accompanied by one or more of the elements Ca, Mg, and K (and Si with or without Al), plots of Cu content against Fe and Mn concentrations gave highly significant positive correlations (Figure 2a,b). (Also note that in each plot all the coatings form a single unified trend despite their wide variations in outward appearance.) In contrast, the Cu in Cu-bearing cell coatings with no detectable Ca, Mg, or K (and with or without Si or Si + Al) showed no significant correlation with Mn or Fe; and in most of these specimens, the Cu level per unit concentration of Mn or Fe was anomalously high with respect to specimens containing one or more of the elements Ca, Mg, and K (Figure 2a,b). Although strongly covariant with Mn and Fe, Cu in cell coatings was independent of Si and Al (not shown).

Viewed in the light of our general knowledge of fundamental biogeochemical processes, these results are tentatively interpreted as follows. Cu (presumably in the form of Cu(II) species) is readily sorbed and coprecipitated by colloidal MnOOH and FeOOH deposits on bacterial cell walls and fibrils and is also sorbed directly by ligands on the surfaces of the cell walls and fibrils. Through the process of specific sorption (surface complexation), Cu is bound strongly and, in large part, covalently to the MnOOH and FeOOH (*2*),

besides being occluded inside these oxyhydroxide deposits owing to coprecipitation, and hence is not displaced by cations such as Ca^{2+}, Mg^{2+}, and K^+ dissolved in the ambient water. In contrast, Cu is bound more weakly (perhaps to a greater extent electrostatically) to cation exchange sites (e.g., $-COO^-$ and $-PO_4^{2-}$ groups) on the biopolymers of the cell walls and fibrils (*16, 24, 26–28, 39*) and is not occluded, and is therefore subject to displacement by the more numerous competing alkali and alkaline-earth cations through the less specific process of mass action (*2*). However, the weak binding sites on the naked biopolymer surfaces greatly outnumber the strong ones on the MnOOH and FeOOH. The result is that in the absence of the competing cations the abundant Cu(II) sorbed to biopolymers masks the association of Cu(II) with MnOOH and FeOOH, whereas in the presence of these cations the bare biopolymer surfaces are selectively swept clean of Cu(II), revealing the association of Cu(II) with MnOOH and FeOOH. Furthermore, the fact that variation in Cu with respect to Mn and Fe is virtually the same for the finer and coarser coatings suggests relative uniformity of oxyhydroxide properties despite the contrast in gross texture; it implies that the "coarse" deposits (not counting Si- and Al-rich ones, which were probably made up of clay crystals) were built up by accretion of multiple fine, thin oxyhydroxide layers similar to one another in physical properties and Cu content and that the Cu in the coarser coatings was scavenged principally by coprecipitation, not by sorption occurring after formation of the coatings.

Uncoated cell walls presented a radically different picture distinguished by *inverse* correlation of Cu with Fe, Al, and Si (Figure 3a–c). In plots of Cu content against Fe, Al, and Si content (Figure 3a–c), all points cluster about the same regression line regardless of whether any of the elements Ca, Mg, and K are present. Also note that the significance of the correlation decreases in the order Al > Si > Fe and that the shape of the regression line which best defines the trend (according to a goodness of fit test) is the same in each plot (semilogarithmic, with Y as a function of log X). These relationships suggest that Al^{3+} ions complexed by ligands such as $-COO^-$ and $-PO_4^{2-}$ groups on cell wall biopolymers (*16, 24, 26–28*) [and possibly on extracellular biopolymers (*38*)] nucleate the precipitation of aluminosilicates (clay minerals) containing Fe; incipient FeOOH coatings on the biopolymers or clay, or both, could also be involved. We infer, moreover, that these deposits interfere with sorption of Cu(II) species such as Cu^{2+} and $CuOH^+$ by competing with them for binding sites; in fact, they probably block many of the binding sites permanently. The effects of these phenomena, however, must be offset to some extent by the tendency of the mineral deposits themselves to sorb Cu (Figure 2a,b). Our observations imply that the uncoated cells are actually coated to some extent with mineral deposits and differ from coated cells only in degree, not in any fundamental way. Thus, the striking difference between element relations in coatings and uncoated cell walls could be ascribed to a difference in the *net* effect of various simultaneously occurring processes (sorption by cell wall polymers, sorption by mineral coatings, the masking of cell wall sorption sites by mineral deposits, etc.) that have jointly produced the observed result.

At this point we may well ask how effectively the scavenging of Cu by cell walls and coatings prevents Cu from crossing the cell membrane and entering the cytoplasm and whether this phenomenon represents a detoxification mechanism—an adaptation to a metal-polluted environment (and, in unpolluted environments, a means of concentrating nutrient trace elements, including Cu, from dilute solution). We must also consider the possibility that uptake of Cu and accumulation of the Cu in cytoplasmic inclusions is an alternative mechanism of detoxification (and micronutrient amassment). Pertinent evidence was obtained by comparing

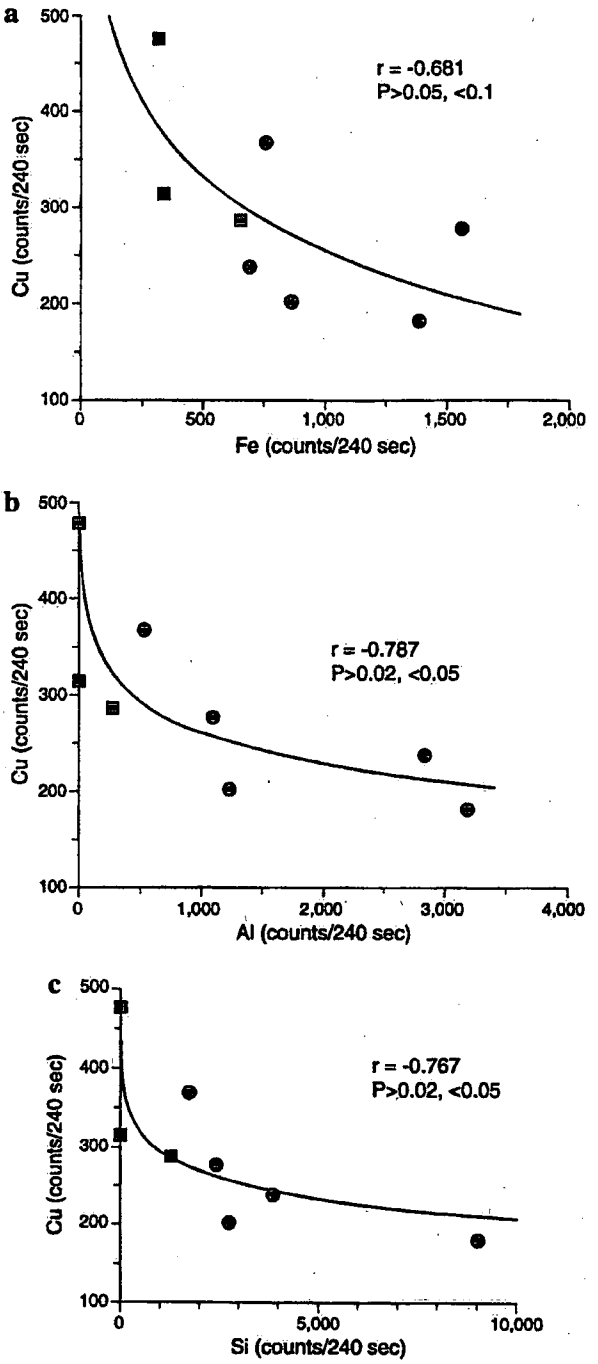

FIGURE 3. Variation of Cu content with respect to Fe (a), Al (b), and Si (c) content in cell walls of uncoated bacteria in Larder Lake sediment. (●): At least one of the elements Ca, Mn, and K is present. (■): Detectable Ca, Mg, and K are absent.

data for the exterior and interior parts of each cell in a series of coated cells containing intact cytoplasm characterized, in some cases, by electron-dense inclusions. The results were highly variable, suggesting coexistence of cells that interact in altogether different ways with heavy metals in their environment. Both the coatings and the inclusions tended to be enriched in Fe and, less commonly, in Mn as well with respect to the associated cytoplasm. Moreover, most inclusions were also enriched in P, implying that they were polyphosphate deposits (*40*), although one Fe-rich inclusion had no detectable P. In some cells the Cu concentrations were highest in the coatings, but in others they were highest

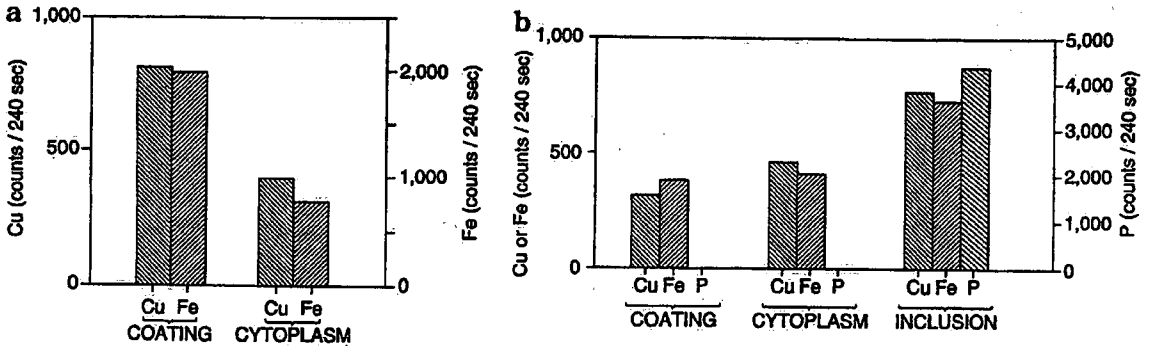

FIGURE 4. Abundances of Cu, Fe, and P in different components of individual bacterial cells in Larder Lake sediment: (a) Cell with coarse, patchy coating (probably FeOOH) enriched in Cu and Fe with respect to the cytoplasm; coating and cytoplasm are both devoid of detectable Mn, P, Si, Al, Ca, Mg, and K. (b) Cell with coarse, even coating and cytoplasm containing an inclusion (probably polyphosphate) enriched in Cu, Fe, and, above all, P with respect to the cytoplasm and coating (neither of which contains detectable P); Si, Al, Ca, and K are present in all three components, but there is no detectable Mn.

in cytoplasmic inclusions, implying the existence of two totally different mechanisms of metal accumulation (Figure 4a,b). In one cell, for instance, the abundances of Cu and Fe varied in the order coating > cytoplasm (Figure 4a), and no inclusion was seen; but in another cell, which contained an inclusion, the order was inclusion > cytoplasm > coating, and P was abundant in the inclusion but undetectable elsewhere (Figure 4b).

These contrasting results may represent two alternative strategies for heavy metal detoxification (*41*–*43*) that evolved in different bacterial species or even in the same species. Soluble Cu species probably differ in their relative tendencies to be sorbed by oxyhydroxide coatings (*2*) or taken up by cells and deposited in cytoplasmic inclusions; perhaps it is for this very reason that both of these unlike mechanisms for immobilizing metals evolved. Possibly widely differing microenvironments created by different species of bacteria in the immediate vicinity of their cells (or differences between living and dead or active and inactive, individuals of the same species) play key roles in the interactions of cells with Cu. In any event, our preliminary observations suggest that uptake of Cu by the cell followed by immobilization of much of it in cytoplasmic inclusions has been a more common and possibly more effective method of detoxification than exclusion of Cu from the cell through sorption by cell coatings.

To the best of our knowledge, this paper represents the first application of modern EDXM and TEM techniques to the systematic quantitative analysis of statistically meaningful numbers of individual bacterial cells, with separate analyses of specific cell components and associated nonliving material in assemblages occurring naturally in sediments. On the basis of our results, moreover, we have been able to draw reasonable, if tentative, inferences about the biogeochemical mechanisms and ecological implications of the observed effects. Processes of the kind envisioned are probably widespread in aquatic environments. In any case, work of this nature opens the way to important but practically unexplored areas of biogeochemical and ecological investigation that are inaccessible by other methods and are far beyond the reach of conventional methods such as bulk analysis. Further research is needed (and, indeed, is now in progress) to amass a more extensive and diverse body of empirical knowledge about interactions of benthic bacteria and sediment constituents with heavy metals and other elements in both natural and experimentally modified environments and to assess the significance of this information for microbial ecology and the problem of pollution prevention and abatement.

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