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Mineralogical and Chemical Characteristics of Biomats from the Mining and Drainage Area

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Abstract: Variations in the mineralogy, chemistry and micromorphology of biomats were studied at four mining disposal and drainage area in Japan. Biomats showed poor crystalline zinc, manganese and iron hydroxides with organisms. Detrital quartz, feldspars, calcite coexist with clay minerals in the biomats. Microorganisms included both prokaryote and eukaryote, especially bacteria, blue-green algae and algae were observed. Bacterial community was covered generally by heavy metals of Zn, Fe and Mn. Heavy metal covered furthermore cell wall of bacteria suggesting accumulations of heavy metal on the surface of bacteria. Heavy metal accumulation indicates that the microorganisms play a role of scavenger during drainage of these elements. The concentration of metallic elements in mine drainage systems might be the most likely factor controlling the accumulation of metals in the biomats. Based on the high efficiency of microorganisms for accumulation of the metal ions, the remediation of heavy metal pollution appears applicable to the types of contaminants in the geological environmental systems.

1. Introduction

Microbial mineral formation evolved early in the Earth's history, have been playing an important role in the genesis of some mineral deposits. Dissolution and precipitation of elements is in part microbially mediated under natural surficial conditions. Microorganisms use elements of C, N, P and S for their growth in this condition (Alexander, 1994). Under present-day polluted environment, bioremediation in contaminated areas is a new field of endeavor, and bacteria are being used for decomposition and absorption of toxic chemicals in soils, groundwater, waste water, sluges, industrial-waste systems, and gases. Microorganisms have play an important role for the bioremediation, particularly to the fixation of a tremendous range of metals and minerals in contaminated areas. There are many examples of microbial remediation in the modern natural environment (Sudo, 1982;

Brady et al., 1986; Leadbeater and Riding, 1986; Tazaki et al., 1990; Tazaki, 1991, 1993, 1994; Skinner and Fitzpatrick, 1992; Vernet, 1992; Markert, 1993).

Microorganisms exhibit a profound ability to bind metallic ions, and this allows cells to serve directly as nucleation sites for initiation process of biomineralization. Oxidation and reduction of metals are caused indirectly by biological processes involving a modification in the redox potential of sediments through consumption or production of oxygen, nitrogen reduction, production of sulphurous hydrogen (Vernet, 1992). The metals deposited in bacterial intra- or extra-cell proceeded to form biominerals with a well-defined structure (Mann et al., 1985, 1987, 1989, 1992; Ferris et al., 1986, 1987, 1989; Mann and Fyfe, 1987, 1988; Tazaki and Fyfe, 1992; Koiwasaki et al., 1993; Tazaki, 1993). In sediments receiving acid drainage from mine tailing and heaps of coal waste, a variety of iron oxides precipitates. The major iron hydroxide is ferrihydrite, goethite and hematite. Electron microscopic studies have shown that bacteria are capable of serving nucleation sites for authigenic formation of these minerals (Tazaki, 1994). The application of electron microscopic techniques to biologic environmental analysis has provided detail information in heavy metal contaminated areas (Beveridge et al., 1983; Beveridge and Fyfe, 1984; Ferris et al., 1986, 1987; Mann et al., 1992; Vernet, 1992).

In this paper, microorganisms for remediation of metal concentration is described for various cases in mining waste disposal and drainage areas, using electron microscopic techniques.

2. Study areas

Omori silver mine now closed, located in Omori, north middle of Shimane Prefecture, occurs in Miocene tuff. Silver-bearing minerals such as argentite, silver, chalcopyrite and galena occurred as disseminate ores and veins (Fig. 1A). Water drained from the mining gallery has a pH of 8.0 - 8.5. Orange colored biomat in the mining gallery is formed on the surface of Miocene tuff.

Homam-zan copper mine now abandoned, located in Adakae, Shimane Prefecture, was once one of the largest and oldest copper mine used to produce chalcopyrite and sulfide minerals with traces of silver and gold. Galena, sphalerite and pyrite were also occurred in dacite and pyroclastic tuff. The geological setting is characterized by rhyolite lava and pyroclastic tuff of Neogene period (Inoue et al., 1982; Toyao, 1985; Yonashiro, 1985). Chalcopyrite-pyrite-quartz mineral assemblage occurred in veins. Through the mining drainage system, polluted water and non-consolidated sediments were continuously transported by underground water into the streams. Fig. 3A shows a general view of an iron seep in Homan-zan mine drainage. This well developed seep has the characteristic of dark-reddish or orange-brownish color indicating an extensive accumulation of iron hydroxide on the bacteria sheaths (see Fig. 3C). Orange colored biomat was formed in mine-tailing ponds, streams and river in the Homan area with a low pH (pH 3.0 - 4.0).

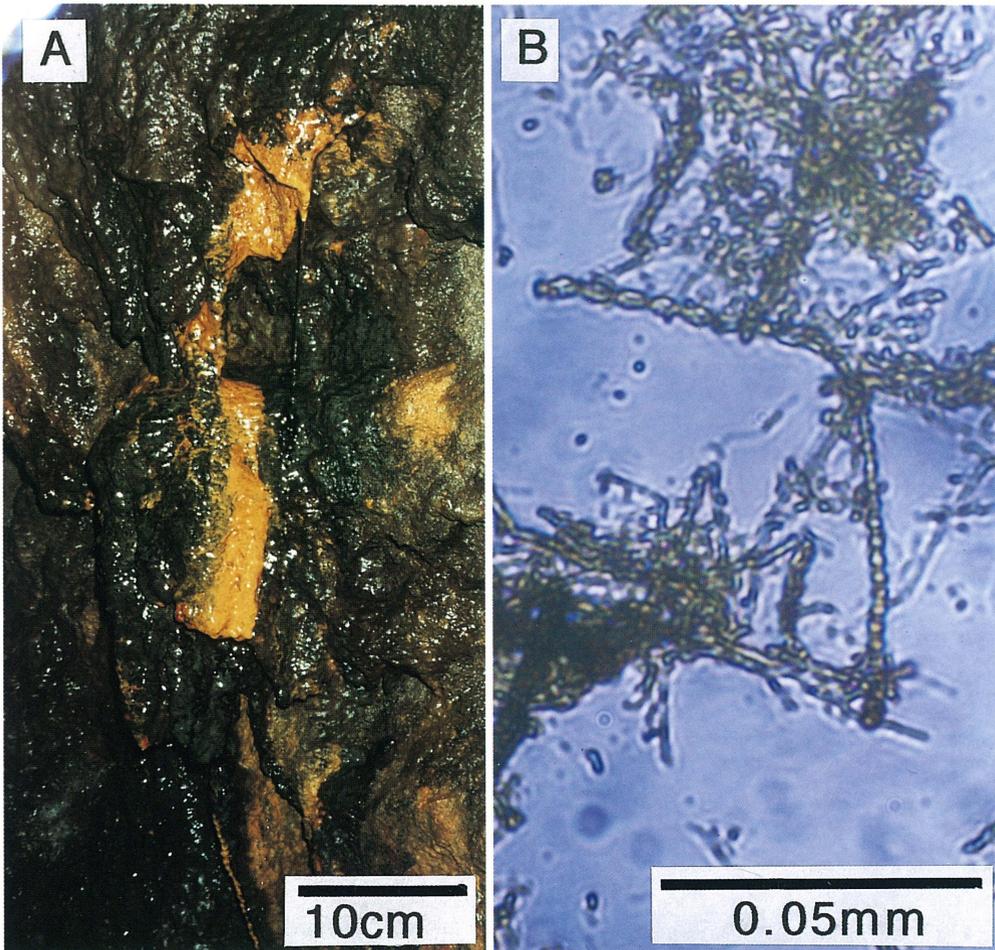


Fig. 1. Soft ochre deposit formed on the surface of Miocene tuff in Omori silver mining gallery (A). Phase contrast light photomicrograph of biomat composed of coccoidal and ellipsoidal algae. The chain-like sheaths are characteristic of iron-fixed *Cyanobacteria* (B).

Near the mining pond, the average pH was around 3.6 (Pires and Tazaki, 1993).

Nakadatsu skarn mine is one of the contact metamorphic deposits, located in Nakadatsu, Fukui Prefecture. Pyrometasomatism by the infiltration and infilling mineralization between zinc-rich high temperature fluid and wall rocks had resulted in the deposition of sphalerite ores. Ore bodies are composed of hedenbergite, galena, sphalerite and pyrite (Yamashita et al., 1988). Black colored biomat in mining gallery was found on the garnet skarn rocks (Fig. 7).

Hishikari gold mine, located in Hishikari, Kagoshima Prefecture, is characterized by Au, Ag-rich epithermal deposit developed on the Quaternary volcanic rocks. Quartz and cryolite veins with gold, silver-bearing minerals associate with the ore deposits. Au, Ag

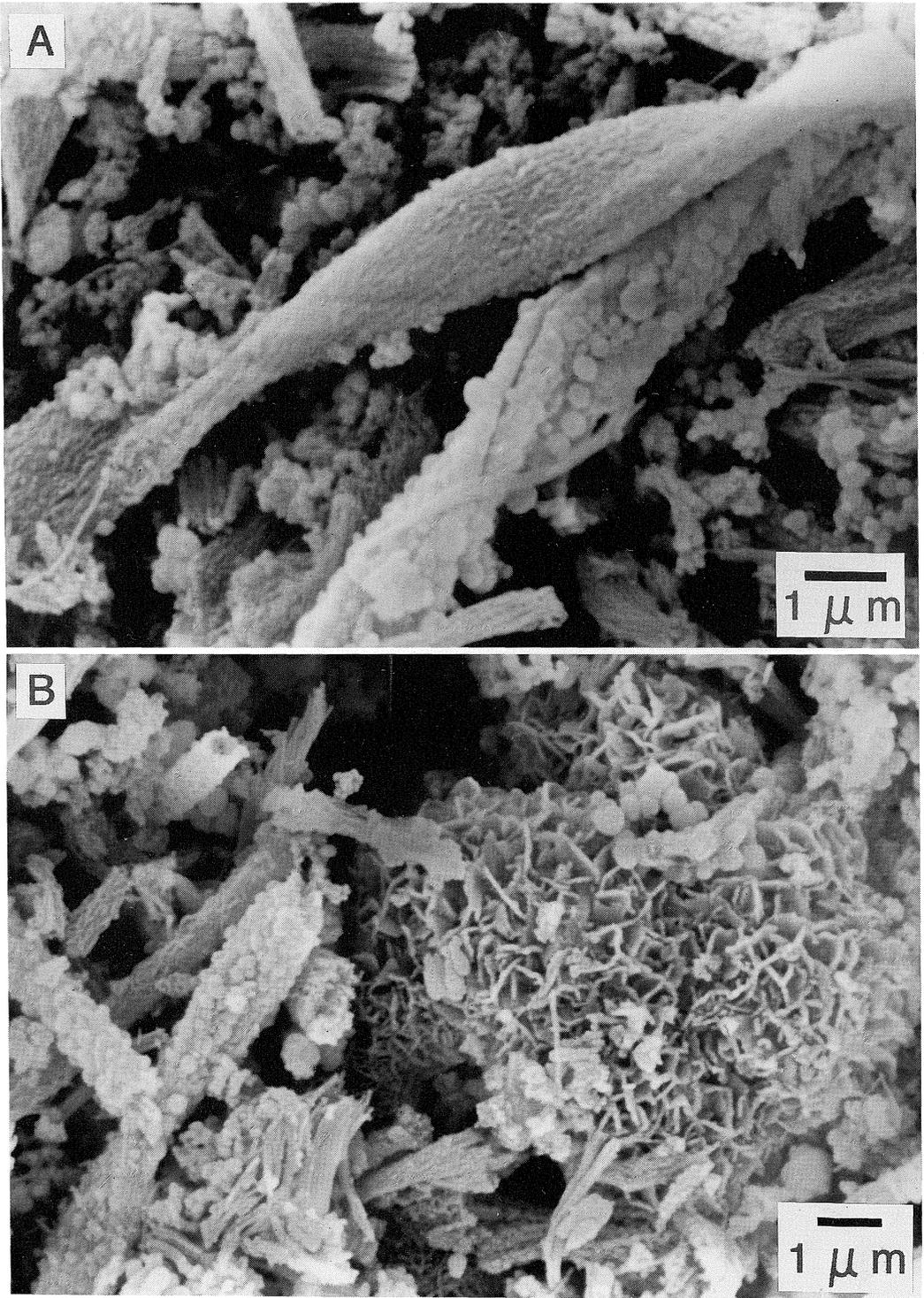


Fig. 2. Scanning electron micrographs of biomat composed of a zinc-concentrate twisted stalk of *Gallionella*-like (A), several tubular sheaths (B). Note the granular masses adhering to stalks.

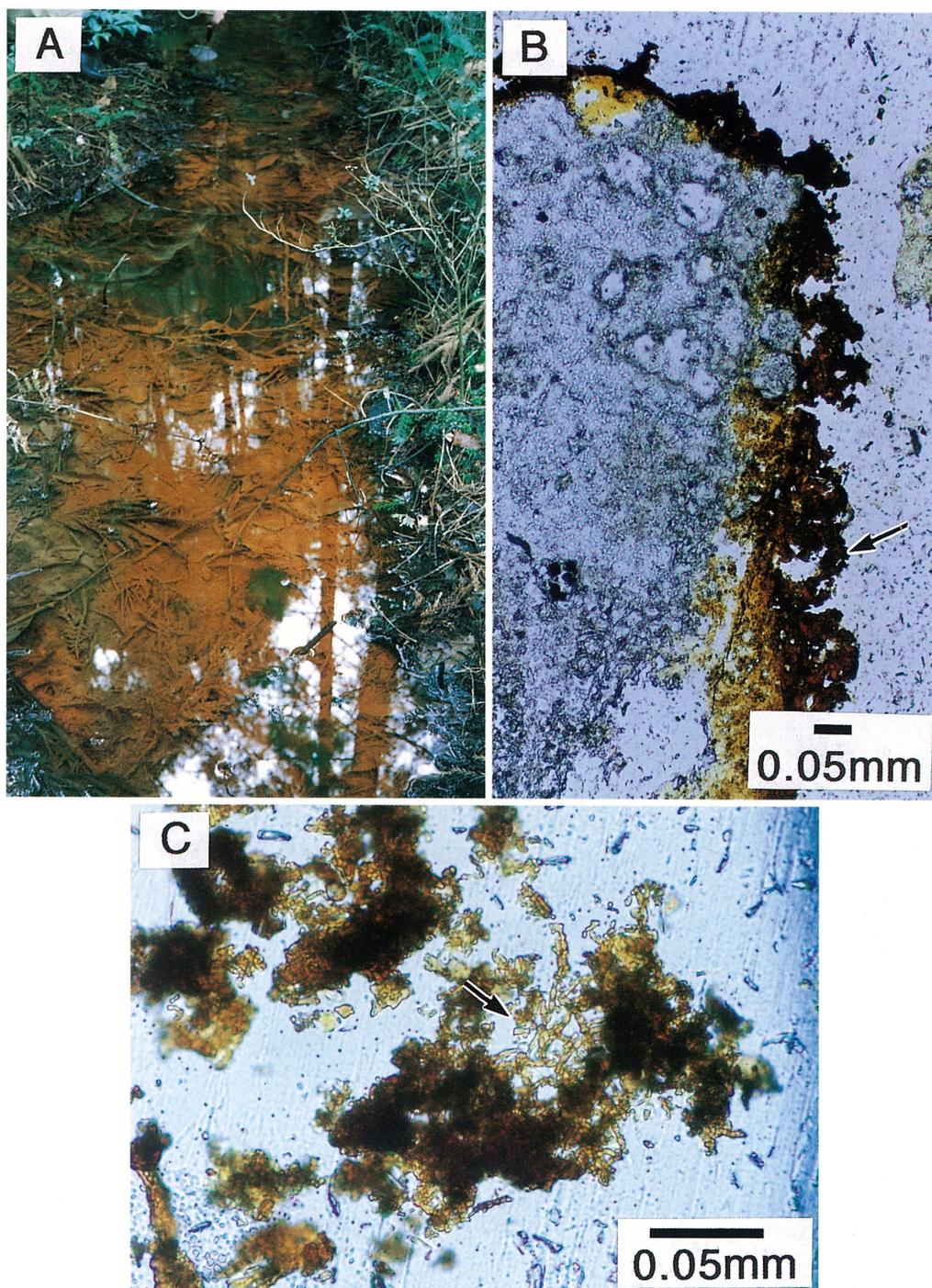


Fig. 3. A photograph showing a general views of an iron seep in Homan-zan copper mine drainage. The well developed seep has the characteristic dark-reddish or orange-brownish color indicating extensive iron accumulation of the bacterial sheaths (A), an optical micrograph of the surface of rock varnish occurring on a substrate collected at the bottom of drainage. Microorganisms adhesive to the surface of rock gravels (B), and an optical micrograph showing the filamentous sheaths from the surface of an oxidized iron deposit on the edge of rock varnish (B, arrow). The filamentous threads on which the iron hydroxides have formed are bacterial in origin. Note the chain-like sheaths (C, arrow).

-bearing minerals include electrum, naumannite, pyrargyrite, chalcopyrite, stibnite, pyrite, quartz, cryolite, calcite and montmorillonite (Sumitomo metal mining, 1992). Brown biomat was formed in the mine-tailing pond and drainage.

3. Materials and methods

Brown, black and orange colored biomats were collected from the nearby area of abandoned mine-tailing ponds, streams and in the mining galleries at Omori silver mine, Homan-zan copper mine, Nakadatsu skarn mine and Hishikari gold mine. The biomat samples were observed with an optical microscope and a phase contrast light microscope, JEOL JSM-220 and JSM-5200 scanning electron microscopes (SEM) equipped with a PV9800 STD energy dispersion system, and a Rigaku X-ray fluorescence analyzer 3134P type (XRF). Mineralogical compositions of these biomats on the slide glass were determined using a Rigaku X-ray powder diffraction (XRD)(Cu-K α radiation).

4. Results

4.1 Omori silver mine

Phase contrast light microscopic observation showed that biomat was composed predominantly of chain-like sheath with characteristic of iron-fixed *Cyanobacteria*. Individual algae shows coccoidal and ellipsoidal morphology (Fig. 1B). SEM observation showed algae having filamentous and twisted structures (*Gallionella*-like) (Fig. 2A). They were frequently covered by large amounts of granular or coccoidal materials. Cross-link colloidal communities were also found as associations with filamentous and twisted algae (Fig. 2B). High concentration of Zn was detected in the biomat. Several tubular sheaths and other zinc-concentrated part seen in the center and right of Fig. 2B were not identified with specific bacteria. Granular masses adhering to stalks also can be seen in Fig. 2B. Table 1 cited the concentration of trace elements in the biomat. The biomat is

Table 1. X-ray fluorescence analysis of bulk sample of biomat from Omori silver mine. The biomat was characterized by enrichment of zinc.

Total counts are over 30,000 cps. -: not detected.

Elements:	Mo	Nb	Zr	Y	Sr	Rb	Pb
	-	1	1	-	91	11	-
Elements:	Zn	Cu	Ni	Cr	V	Ba	
	>11000	45	12	-	-	-	(cps)

characterized by enrichment of Zn element with trace amounts of Sr, Rb, Cu and Ni.

Biomat in the mining gallery were examined by XRD. To compare the biomat with surrounding rocks and sediments, shale, green clay, tuff and soil were also examined (Table 2). Major minerals detected in the biomat were illite/smectite interstratified minerals and kaolinite, whereas a large amount of illite, quartz with small amounts of feldspars and illite/smectite interstratified minerals were detected in the biofilm sample. The XRD patterns of biomat and biofilm showed broad weak reflections and high background, suggesting a presence of poorly crystallized materials. Shale consists of illite/smectite interstratified minerals, chlorite, kaolinite with small amounts of quartz, feldspars and olivine. A large amount of quartz, feldspars and pyroxene was detected in the tuff. Illite/smectite interstratified minerals, muscovite, kaolinite, quartz and feldspars were major constituent of the green clay sample. The soil sample contains mainly illite/smectite interstratified minerals and quartz.

Table 2. Mineral assemblage of rocks, soils, clays and organic matter samples from Omori silver mine.

I/S: illite/smectite interstratified minerals, S: smectite, Mu: muscovite, I: illite, Ch: chlorite, Ka: kaolinite, Q: quartz, F: feldspars, Ol: olivine, Px: pyroxene, ++++: abundance, +++: common, ++: small amounts, +: trace.

	I/S, S	Mu	I	Ch	Ka	Q	F	Ol	Px
shell	++			++	++	+	+	+	
black biomat	+				+				
black biofilm	+		+++			++++	++		
black soil	+++					+++			
tuff	+					++++	++		++
green clay	++	++			+	++	+		

4.2 Homan-zan copper mine

Optical microscopic observation showed that the gravels collected at the bottom of drainage were covered by dark-reddish or orange-brownish viscous materials (Fig. 3B). The biomat consists predominantly of filamentous algae, twisted algae and diatoms (Fig. 3C). The diatom species were identified as *Aulacoseira* sp. and *Navicula oblonga*. Cu-Fe heavy metals were coating around the surface of gravels. Fig. 3C shows the filamentous sheaths from the surface of an oxidized rock varnish (Fig. 3B, arrow). SEM-EDX examination revealed that the most of filamentous algae showed a teardrop morphology, the surface of which was coated by granular materials (Fig. 4A). EDX analysis showed

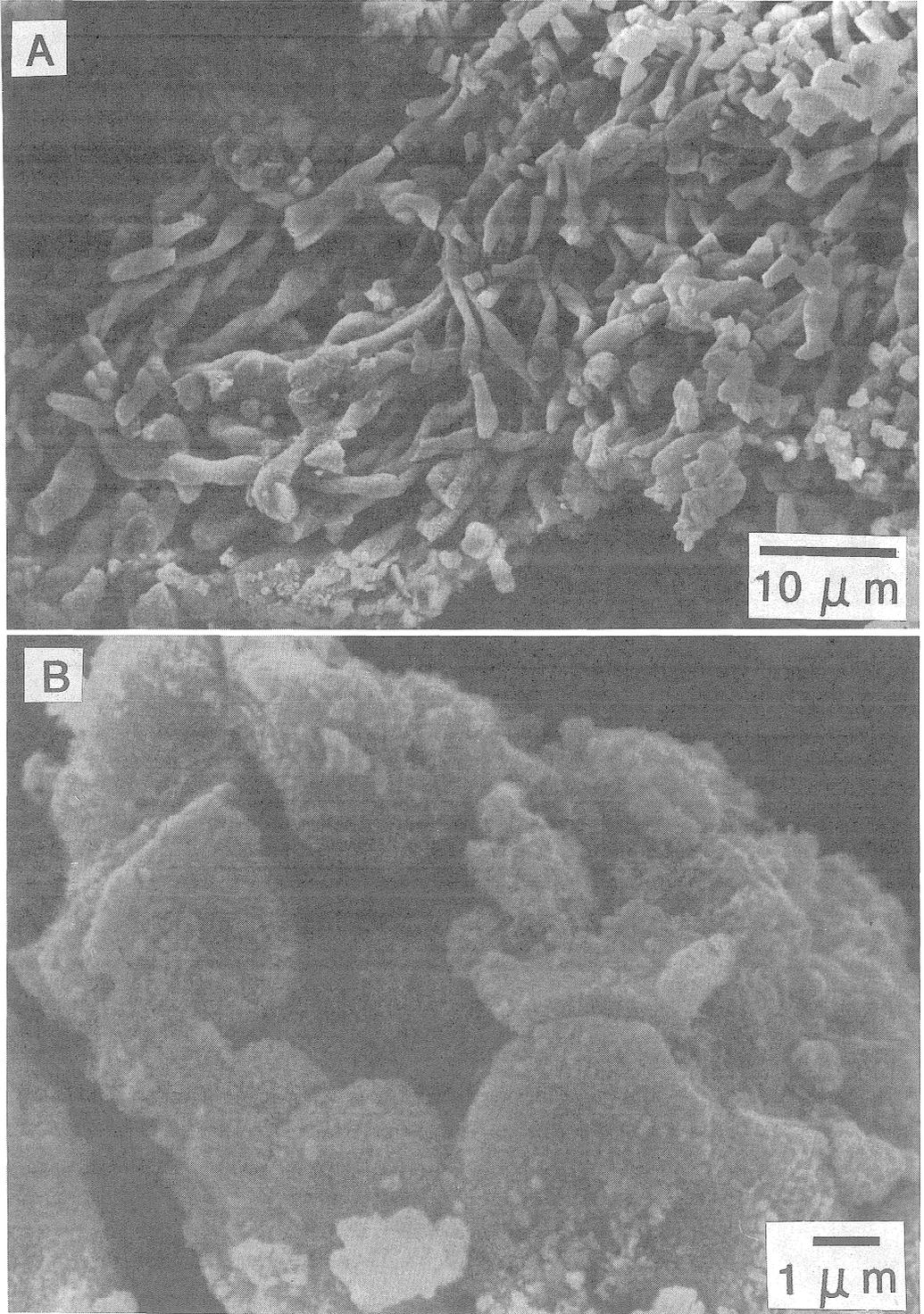


Fig. 4. Scanning electron micrographs of biomat composed of iron-precipitating bacteria from Homan-zan copper mine drainage, showing filamentous microstructures (A). Scanning electron micrograph of the friable, iron-rich, surficial crust from Homan-zan copper mine drainage, showing ferrihydrite on a clay-like matrix (B).

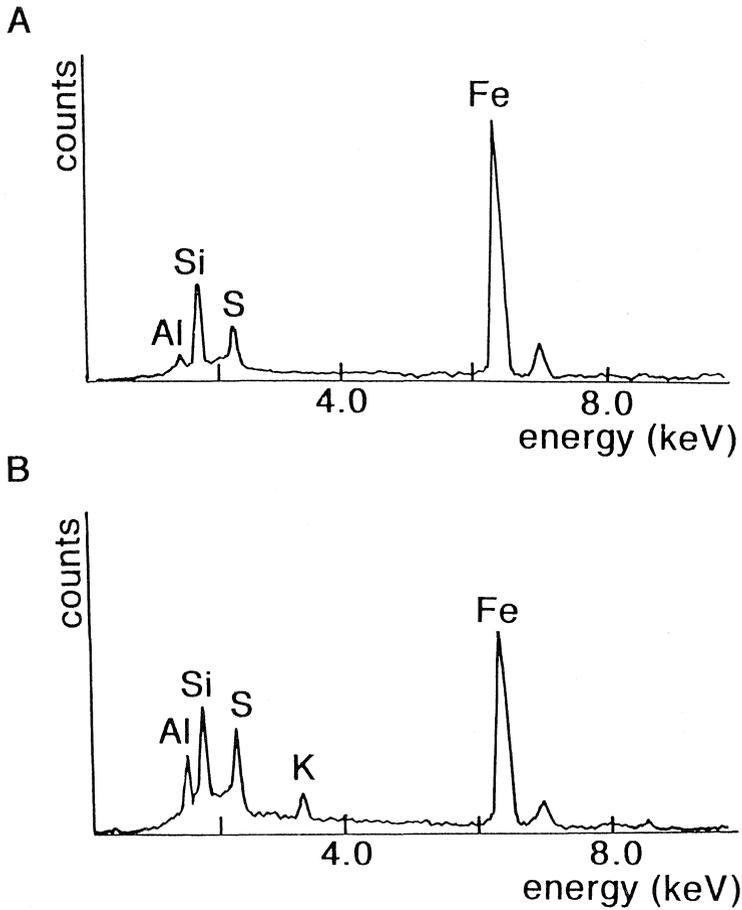


Fig. 5. Energy dispersive analysis (EDX) of bulk sample of filamentous algae shown in Fig. 4A, and the surficial crust shown in Fig. 4B, showing a high concentration of Fe and low concentration of Al, Si, S and K.

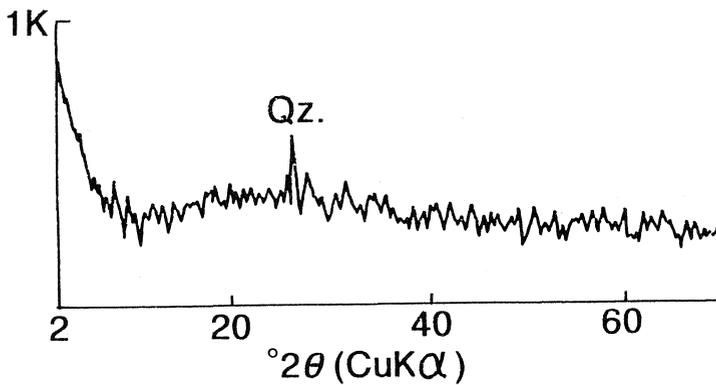


Fig. 6. X-ray powder diffraction pattern of bulk samples of an iron seep from Homan-zan copper mine drainage, showing mainly quartz peaks with a high background. Qz. : quartz.

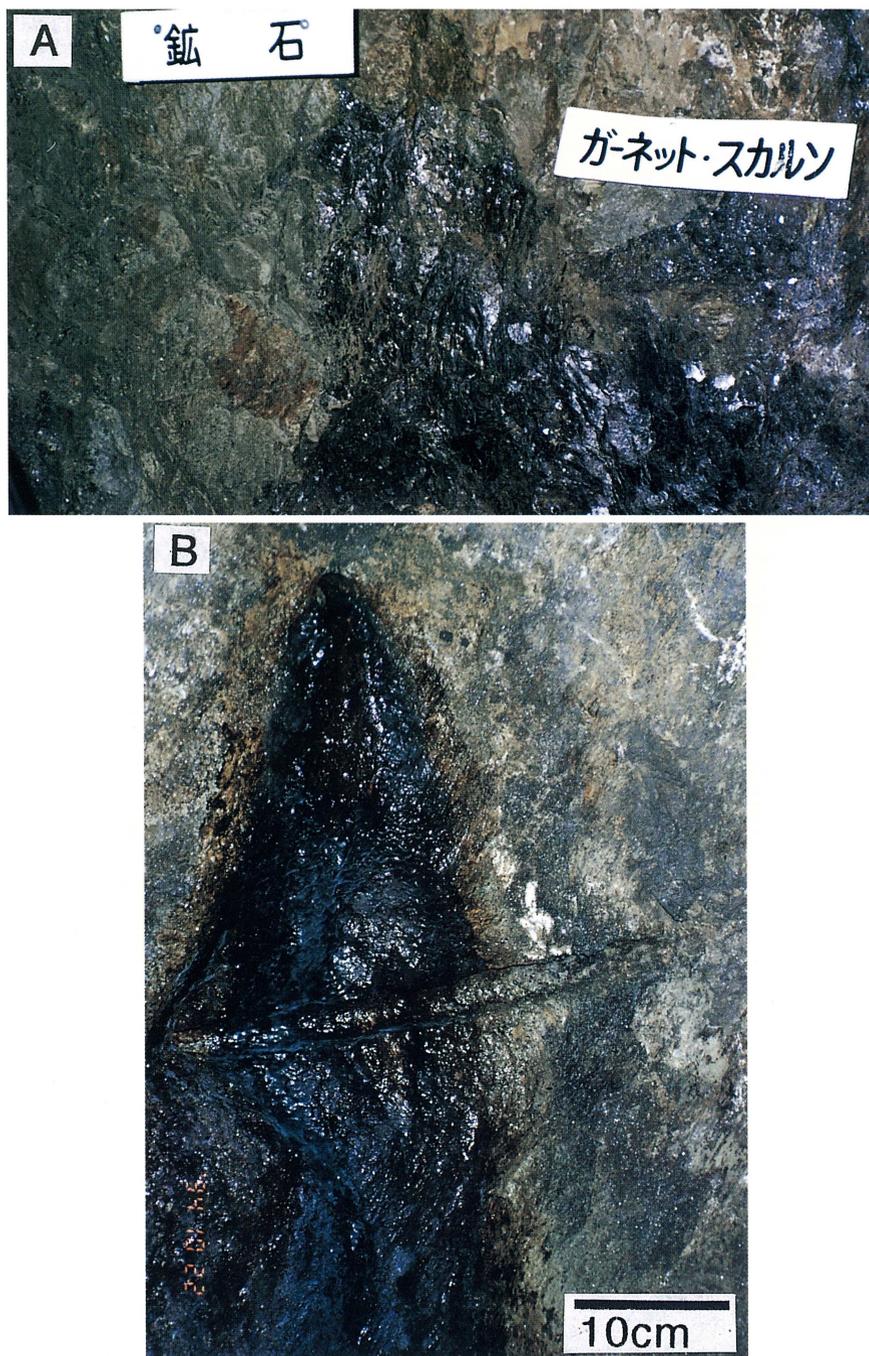


Fig. 7. Soft black biomat (B) formed on the surface of garnet skarn rocks in the Nakadatsu skarn mining gallery (A).

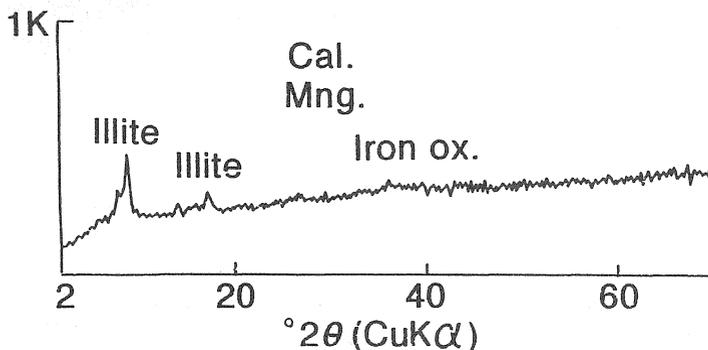


Fig. 8. X-ray powder diffraction pattern of bulk sample of biomat on the rock surface from Nakadatsu skarn mine, showing the peaks of illite, calcite and iron oxide minerals.

Cal.: calcite, Mng.: manganese oxides, Iron ox.: iron hydroxide minerals.

that these granular materials were composed of a high concentration of Fe with low concentration of S, Al, Si and K (Fig. 5), suggesting a presence of filamentous iron-fixed bacteria. Fig. 4B shows the friable, iron-rich, surficial crust from Homan-zan mine drainage, showing ferrihydrite on a clay-like matrix.

The XRD pattern obtained from the biomat showed predominant quartz with trace amounts of poor crystalline hematite, magnetite and akaganeite (Fig. 6). Chemistry, measured by ICP, of the stream water where biomat was found showing high concentration of S (20.42 ppm), Fe (4.49 ppm) and Al (8.12 ppm) with low content of Mn (0.22 ppm), Cu (0.05 ppm), Cr (0.05 ppm), Ni (0.03 ppm) and Ti (0.01 ppm) (Pires and Tazaki, 1993). The enrichment of S and Fe in the streams around the Homan area indicates an active metal accumulation by bacteria.

4.3 Nakadatsu skarn mine

The XRD pattern of bulk biomat from mining gallery (Fig. 7) showed strong reflections of illite at 10 Å and 4.9 Å and broad weak reflections of Mn-rich calcite and iron oxide minerals (Fig. 8). The pattern suggests the presence of carbonate minerals and iron oxide minerals having small crystal size with disorder structure. Some weak reflections at 10 Å, 7 Å, 4.7 Å and 1.9 Å might suggest a presence of todorokite, but it is difficult to identified clearly in the mixture samples because of low crystallinity.

SEM examination revealed that biomat was composed of algae which shows filamentous and teardrop microstructures with fibrous network materials covering on the surface (Fig. 9A and B). Fig. 9B is a SEM photograph of *Leptothrix ochracea*-like sheath. EDX analysis showed high concentrations of Mn with Ca in the sheaths (Fig. 9C). The fibrous network materials are rich in manganese on the sheath. Several minor elements,

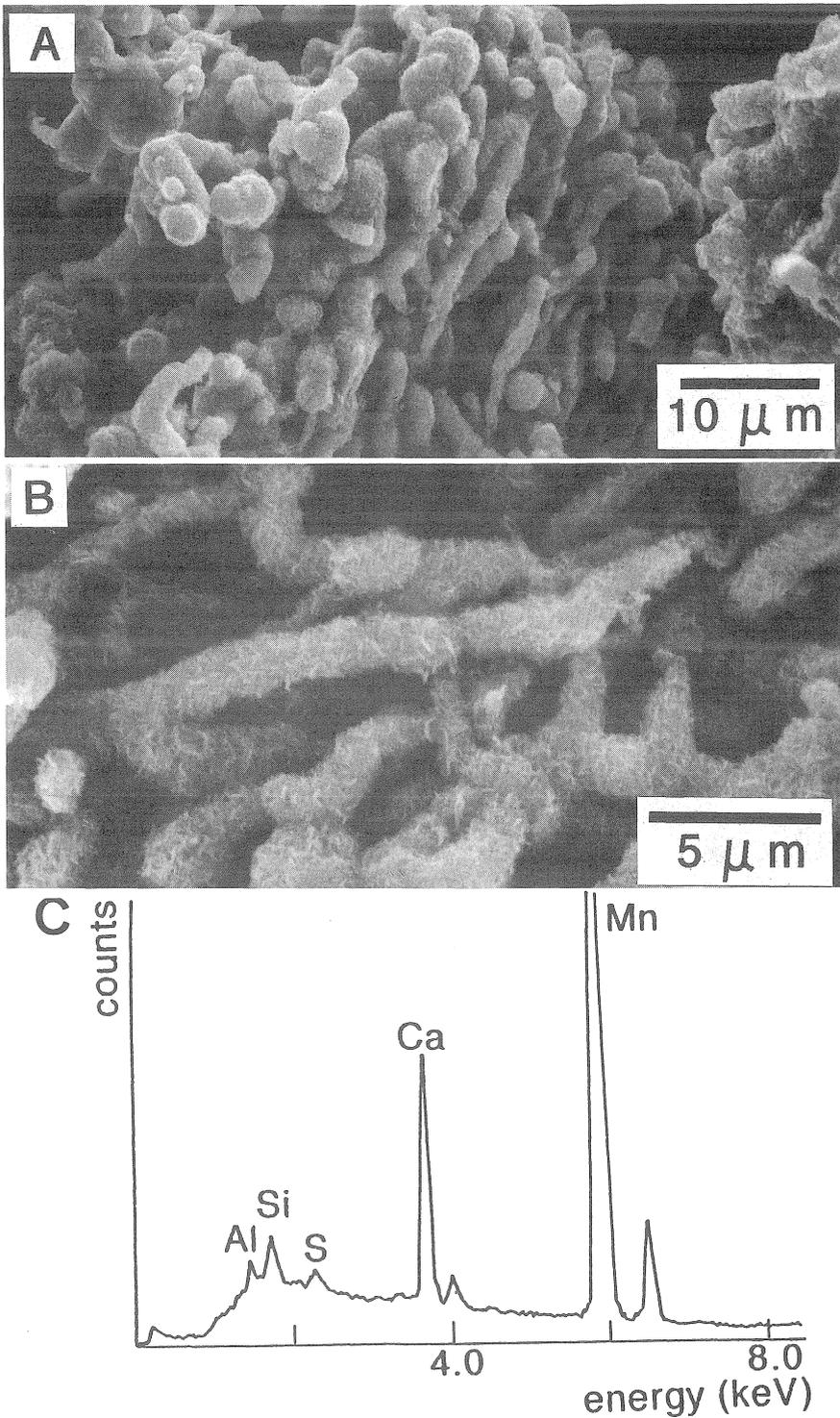


Fig. 9. Scanning electron micrographs showing manganese-precipitating bacteria with filamentous and teardrop microstructures (A). *Leptothrix ochracea*-like sheaths from the same sample as Fig. 7B, showing the presence of granular manganese precipitates on the sheaths (B). EDX analysis showed high concentrations of Mn with Ca in the sheaths (C).

Al, Si and S in the sheath were also detected (Fig. 9C).

4.4 Hishikari gold mine

XRD analysis showed that biomat contained smectite, kaolinite and quartz as major components with small amounts of iron oxide minerals, feldspars and cristobalite (Fig. 10). The reflections at 10 Å, 7.8 Å and 4.7 Å are also suggestive of the presence of poorly crystallized rancieite (Ca, Mn) Mn₄O₉·3H₂O. A high background suggests the presence of amorphous or organic materials.

SEM observation showed biomat comprised spherical or granular shaped algae, generally as aggregates (Fig. 11A and B). Some granules attached to the larger spherical bodies (Fig. 11B, arrow). EDX analysis revealed that microstructure of spherules was rich in Fe and S (Fig. 11C). A high concentration of Al, Si, K and Ca suggests the composition of clay minerals and quartz with organic matter as revealed by XRD result. Traces of Na, Mg and Ti were also detected in the biomat.

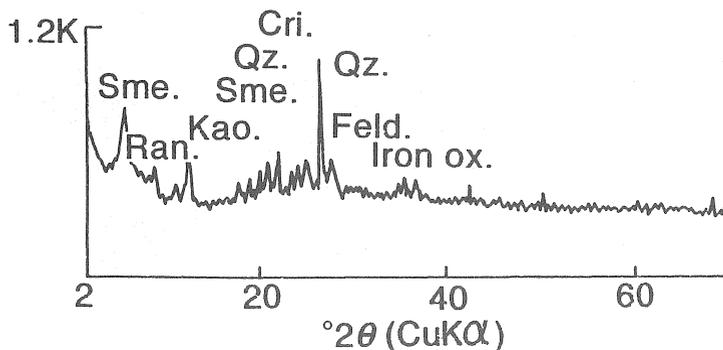


Fig. 10. X-ray powder diffraction pattern of bulk samples of biomat at the bottom of drainage from Hishikari gold mine, showing the peaks of quartz, kaolinite, smectite, feldspars, rancieite and iron oxide minerals. Sme.: smectite, Ran.: rancieite, Kao.: kaolinite, Qz.: quartz, Cri.: cristobalite, Feld.: feldspars.

5. Discussion

Biomats are microbial communities densely entangled with their mineral substrate and organized in response to the sedimentary or geologic environment by physical processes and by the products of biotransfer (Krumbein, 1979). They serve as barriers, traps, filters, sites of biotransfer, and reservoirs thereby producing and structuring the inorganic substrate. This development is often associated with biotransfer within the mats (Leadbeater and Riding, 1986). Biomats are extremely wide-spread biological systems, however, few bacteria species, such as *Cyanobacteria* (*Microcoleus chthonoplastes* and *Oscillatoria* sp.), are commonly observed. The differences in biominerals and chemical compositions of biomats are related to differences in not only microorganisms, but also the

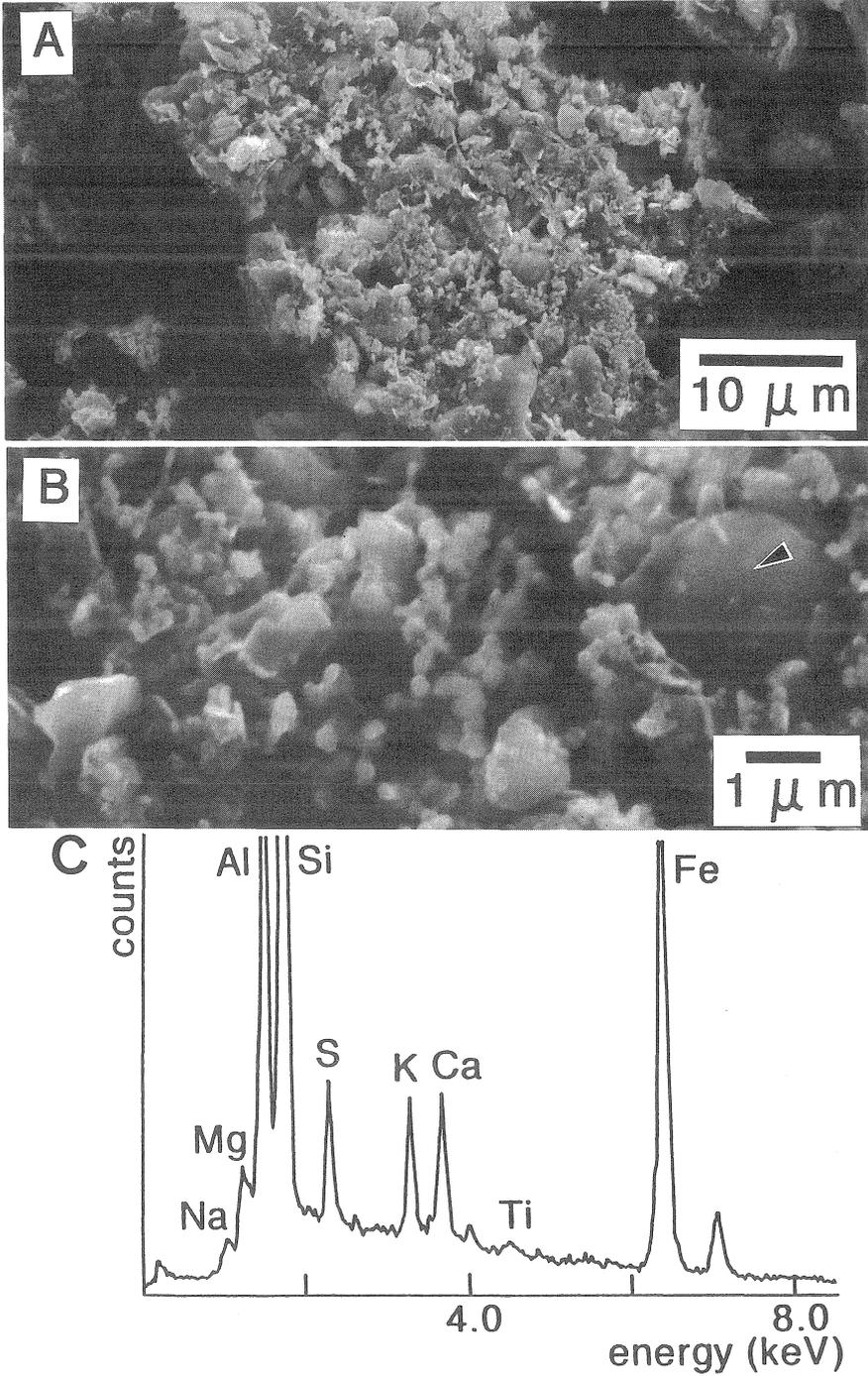


Fig. 11. Scanning electron micrographs and EDX analysis of the biomat from Hishikari gold mine, showing microbial structure of spherical and granular algae rich in Fe (A), and B is a close-up of A. Note that larger spherical bodies have granules (arrow). Polygonal grains are clay minerals probably admixed with organic matter.

ore minerals present. Various minerals produced within the mat systems precipitate during biotransfer processes (Tazaki, 1994). The processes are related to (a) their energy metabolism; (b) inorganic environment; (c) the climatic situation; (d) the water supply; (e) the chemical composition; (f) their internal structure and species composition (Krumbein, 1973). Laboratory and field data on pure cultures and microbial mats demonstrated that gold, silver, and copper are precipitated under bacterial control (Beveridge, 1978; Dexter-Dyer, 1983; Dexter-Dyer et al., 1984).

Biotransfer (trapping, enriching, absorbing, precipitating) in mining biomats have been revealed in this study. Biomats have controlled biotransfer processes and the biogeochemical cycles in wide-spread ore deposition systems in mines. The most likely factor controlling the growth of biomats during heavy metal accumulation might be the concentration of elements in mine drainage systems. The biomineralization of heavy metals appears applicable to the remediation of heavy metal contaminated areas.

6. Conclusions

The mineralogy, chemistry and micromorphology of biomats from industry mines (silver, copper, gold and zinc mines) in Japan have been examined using electron microscopic techniques. Important conclusions from this study are:

1) The mineralogy of biomats shows generally the mixture of poorly crystallized Zn, Mn and Fe-bearing materials and organisms. Other minerals such as quartz, feldspars, calcite and clay minerals are also found in the biomats.

2) Microorganisms identified by their morphologies include both prokaryote and eukaryote, most of them are *Cyanobacteria* (blue-green algae) and algae. They show filamentous, teardrop, coccoidal and spherical structures, generally covered by heavy metals of Zn, Fe and Mn.

3) Biomat is rich in Zn in Omori silver mine, rich in Fe in Homan-zan copper mine, rich in Mn in Nakadatsu skarn mine and rich in Fe in Hishikari gold mine, respectively. The difference in biominerals and chemical compositions may be related to not only microorganism species and but also concentration of element in mining drainage systems.

4) The biomineralization of heavy metals by microorganisms in mine industry drainage shows that bacteria appears applicable to the bioremediation of heavy metals contaminated areas.

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