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メタデータ	言語: eng
	出版者:
	公開日: 2022-12-05
	キーワード (Ja):
	キーワード (En):
	作成者:
	メールアドレス:
	所属:
URL	https://doi.org/10.24517/00010383

Clay Science ***14***, 65–79 (2009)

METAL UPTAKE IN BENTHIC ORGANISMS AND ARGILLACEOUS SEDIMENTS AFFECTED BY MINE DRAINAGE IN OGOYA, ISHIKAWA, JAPAN

KEELY BRANDON, KAZUE TAZAKI and MASAYUKI OKUNO

Division of Earth Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma, Kanazawa, Ishikawa 920-1192, Japan

(Received March 31, 2009. Accepted June 8, 2009)

ABSTRACT

A unique ecosystem composed of liverworts, diatoms, bacteria and argillaceous sediments is able to thrive in spite of acidic, sulfur and heavy metal contaminated water below the abandoned Ogoya Copper Mine in Ishikawa, Japan. Sample analyses by optical microscope, ED-XRF, XRD, SEM, TEM and FE-TEM revealed that Fe and Cu were taken up by benthic organisms and sediments. Metal uptake and precipitation, including adsorption by clay minerals, improves the water quality for surrounding organisms. Liverworts shelter diatoms and bacteria, also trapping sediments and precipitates that would otherwise be washed away. Meanwhile bacteria are involved in metal accumulation and converting toxic aqueous metals to minerals. Liverworts displayed Cu and occasionally Fe and Zn content. Clay sediments were enriched in Fe and sometimes S, with traces of Ti, Mn, Cu, Zn, and occasionally As, Br, Sr and Zr. Based on XRD analyses, chlorite, mica minerals, feldspar, quartz and chloritoid were predominant in the < 2 μm fraction of the river sediments, along with traces of the sulfate minerals melanterite, chalcanthite and despujolsite. Mine drainage has compromised the health of surrounding communities and damaged river ecosystems. Clarification and optimization of natural metal uptake processes and the fate of mine-related elements could hold keys to sustainable remediation methods for use in the Ogoya mine area.

Key words: Ogoya copper mine, Metals, Mine drainage, Clay minerals, Liverwort

INTRODUCTION

Since the late 1800s and continuing well beyond the 1971 closure of Ogoya copper mine, acidic, metal laden, sulfur-rich water has been emitted from underground mine shafts into upper reaches of the Gotani river in Ishikawa, Japan. Pyrite (FeS_2), chalcopyrite ($CuFeS_2$), galena (PbS), and sphalerite (ZnS), are present in the Ogoya mine, and these minerals are associated with acid mine drainage (Gray, 1997; Kishigami et al., 1999). More detailed geological information is compiled elsewhere (Kishigami et al., 1999; Sato and Tazaki, 2000; Nakanishi et al., 2004). Acid mine drainage (AMD) is a major environmental problem around the world, contributing greatly to the up to 1 million tons of heavy metals released into water bodies every year by human activity (Schwarzenbach et al., 2006). It causes environmental damage and health issues in affected areas. Like their global counterparts, Ogoya residents have borne the long-term effects of the area's mining on the environment and agriculture, and historically some have even been affected by mining-related health problems including osteomalacia. Caused by Cd damage to bones, this and other diseases have been linked to mining areas in various countries (Sato and Tazaki, 2000; Tatsuta, 2006). Furthermore, the environment of the once pristine rivers has been severely compromised, and fish have yet to return to many reaches of the river, although mining activities concluded nearly forty years ago. In fact, a national study of water quality in rivers throughout Japan showed that the Kakehashi river, which Gotani River feeds into, is one of Japan's three most polluted rivers, with the highest Cu content and third highest content of Zn, Pb and Cd of any river in the study (Tada et al., 1983).

Remediation of the Gotani river is needed to restore a healthy ecosystem that supports fish and doesn't present health hazards to local residents. Cleanup methods which focus on a single aspect of water quality, such as pH, can have unexpected negative side effects. In order to avoid this problem, this study attempts to provide a basic understanding of natural processes occurring in one severely contaminated area, and interactions between the site's benthic organisms, drainage water and sediments. Some of these processes could enhance remediation efforts while others may hamper them, and a holistic view is useful for designing effective natural remediation programs in Ogoya. Any identified beneficial processes should be optimized, and those that prove to be a hindrance should be controlled.

A drainage ditch receiving direct, untreated mine discharge was chosen for the study. The area does support a limited benthic community, including a dense carpet of liverworts, a few species of pennate diatoms, and other microorganisms. The drainage is characterized by high S content, low pH and unusually high levels of the metals Cu and Zn. Benthic sediments tended to accumulate on and around the liverwort carpet, and varied from sandy to fine grained. Previous studies in the Ogoya mining area indicated that biomats accumulated the metals Mn, Fe, Cu, Zn, Cd and Pb, and that diatoms may act as bioindicators of the mine drainage (Sato and Tazaki, 2000; Nakanishi et al., 2004). Multiple studies have investigated interactions between clayey sediments and metals in the system (Sato and Tazaki, 2000, 2001); however little if any research has focused on this particular site, or on the associations between argillaceous riverine sediments and benthic organisms in Ogoya. As a preliminary investigation, this study provides a semi-quantitative analysis of AMD-associated elements (S, Fe, Cu and Zn) and their major sinks within one stretch of the Gotani River. Such knowledge enables a more enlightened plan for further research and remediation efforts, and clarifies what aspect of the ecosystem is most important for the removal of a certain polluting element.

MATERIALS AND METHODS

Samples

Samples of bryophytes, argillaceous sediments and water were taken from Gotani River and its tributary (Fig. 1) at a major discharge pipe, upstream of the discharge (where bryophyte presence was sparse), and at various points downstream. In and below the drainage pipe, an aquatic bryophyte formed a carpet, completely covering wide areas of the streambed (a, f). Morphologically it is a leafy liverwort (g), and individual clumps (b) grew together into mats (f). In the diagram, bryophyte coverage is indicated by green shading of the riverbed, and sampling points are indicated by light green stars. Red circles indicate points of discharge of groundwater to the river. The large circle represents a major point of mine drainage, below which the riverbed is covered by bryophytes.

Methods

Water quality readings of pH, EC, Eh, DO and water temperature were taken on site in spring, summer and fall sampling periods. Measurements were made just above the major drainage pipe, both inside and at the mouth of the pipe, and at several points in the tributary and main river below the point of discharge (Fig. 2). In conjunction with each field observation, samples of water, argillaceous sediments and liverworts were taken to the laboratory for further observation and analyses. Comparative samples were taken from the main river in the area upstream of the confluence with the mine drainage affected tributary. Much of this water comes from a natural stream flowing from a ski area at the top of the mountain, and it is relatively clean.

River water and liverworts were placed in aquariums to keep live samples available and to observe changes in water quality and liverwort metal content over time. Water and liverworts were mounted and stained with 4',6-diamidino-2-phenylindole (DAPI) for observation by optical microscopy using the Nikon Optihot-2, equipped with ultra-violet (UV) and green excitation (G2A) filters. Dehydrated water samples and powdered samples of liverworts and sediments were analyzed by energy dispersive X-ray fluorescence (ED-XRF) using the JEOL Element Analyzer JSX-3201 at 30.0 kV, 0.9 mA for 900 live seconds. A smaller subset of the samples was analyzed by X-ray powder diffraction analysis (XRD) using the Rigaku X-ray Diffractometer RINT-1200. Further portions of the bryophyte were freeze dried after a preparatory 11 rounds of centrifugation with increasing concentrations of ethanol followed by three rounds with T. Butyl alcohol). Samples were mounted on carbon stubs with carbon tape and coated with carbon using the JEC-520 Carbon Coater machine. Finally, they were observed by scanning electron microscopy and analyzed by energy dispersive X-ray (EDX), using the JEOL JSM-5200LV Scanning Electron Microscope (SEM) equipped with Energy Dispersive X-ray Spectroscopy (EDX) EDAX 9800. Additional sample stubs were prepared using naturally-dried liverworts. Detailed EDX analyses of liverworts, sediments and diatoms were averaged to summarize the findings. A small portion of liverwort was taken from an aquarium with no sediments, crushed with a mortar and pestle, and diluted with distilled water before being mounted on Transmission Electron Microscopy (TEM)/Field Emission TEM (FE-TEM) microgrids for analysis. Initial analyses employed Cu microgrids. For all other analyses, microgrids composed of Mo were chosen in order to more accurately assess Cu content. Additional samples were prepared by cutting small pieces of liverwort with a razor and mixing with water from the original sample before mounting. Samples were observed using the JEOL-2000EX at an accelerating voltage of 160 kV and the JEM-2010FEF.

RESULTS

Water Quality

Water quality varied significantly from point to point at the study site, and seasonally, but trends were consistent throughout the seasons. Higher overall pH and lower overall EC could be attributed to higher water flow (from snowmelt and rain) and subsequent dilution. Average values were calculated for representative points (Fig. 2). The lowest pH readings and the highest EC readings (pH 4.0–4.5; EC 0.25–0.37 mS/cm) were at and near the point of initial output (d) while water coming from upstream (a) was somewhat closer to neutral (pH 5.3–7.0) with lower EC (0.05–0.25 mS/cm), with points in between showing gradation (b, c). Several meters downstream after mixing of the water (e–h) the pH was consistently between 4.5 –5.0 and the EC between 0.1–0.3 mS/cm. At the point where the drainage ditch meets the Godani River, there is a distinct difference in drainage water (i–j: pH 4.7–5.1, EC 0.1–0.3 mS/cm) and relatively clean upstream water (l: pH 6.7–7.6; EC 0.02–0.09 mS/cm), with intermediate readings where the waters mix (k: pH 5.1–6.6; EC 0.08–0.3 mS/cm). Measurements of water in areas upstream points y and z corresponded

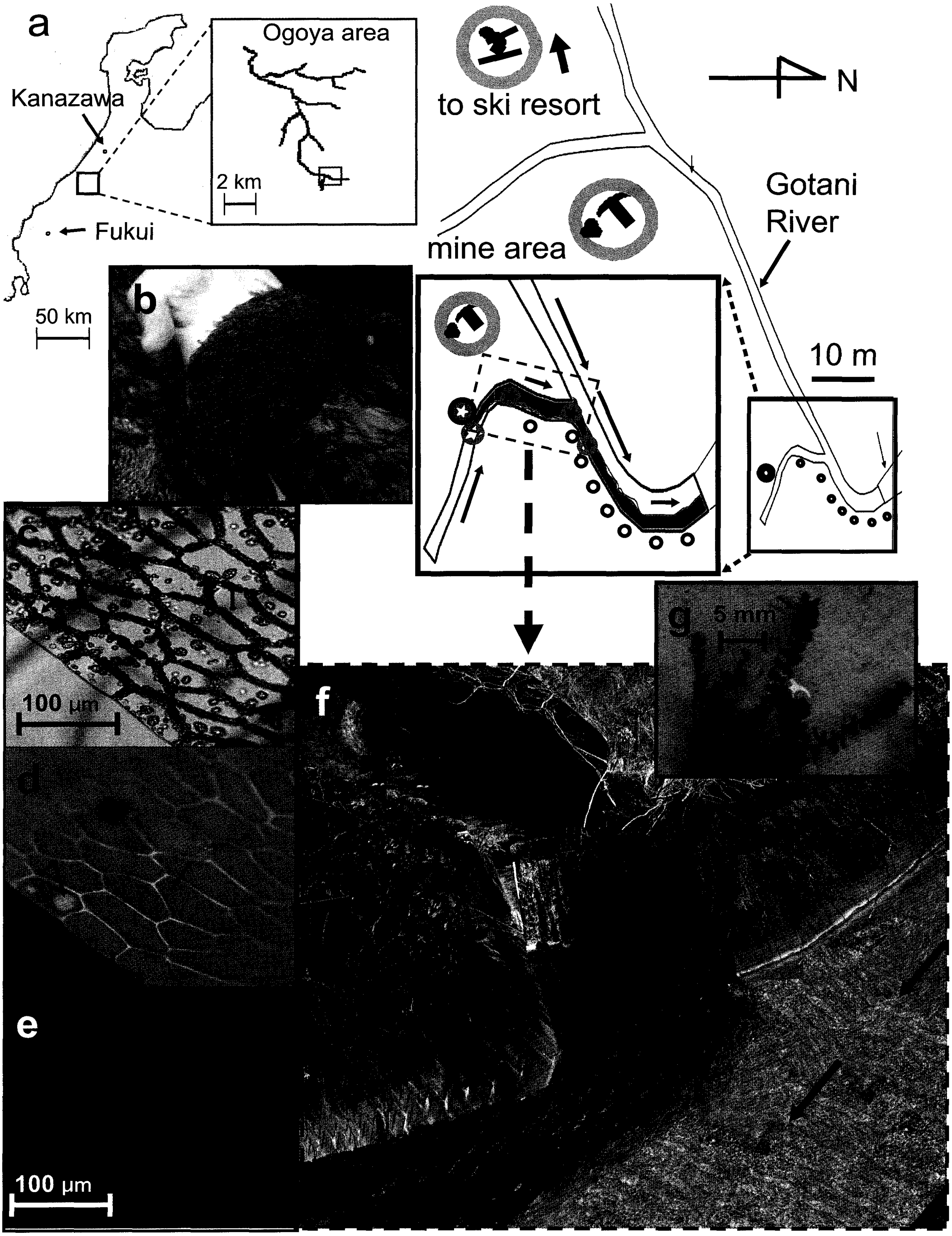

FIG. 1. Overview of the study site and sampling points below Ogoya Copper Mine, Ishikawa, Japan (a, f) with photographs and optical micrographs of liverworts (b–g). Bryophyte coverage is indicated (a) by green shading of the stream, and bryophyte sampling points are indicated by green stars. Red circles indicate points of discharge of groundwater to the river. Below the first drainage point (large circle) the riverbed is covered by bryophytes. Microscopic images of the bryophyte reveal oil bodies (c, arrows) indicating that it is a liverwort, as well as minerals forming within and on some cells (d). Most chloroplasts in healthy cells cluster around the cell edges (e)

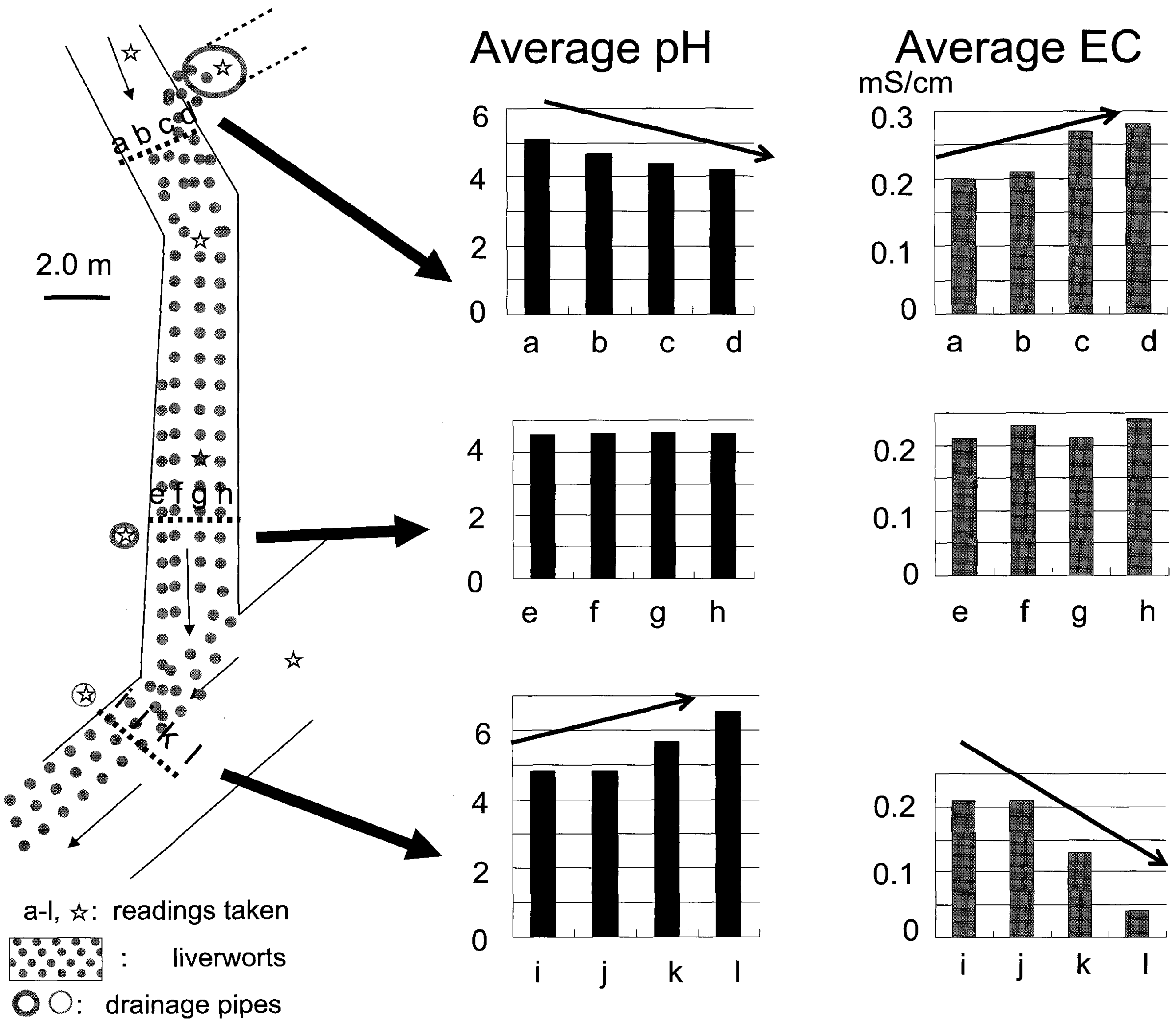

FIG. 2. Average pH and EC readings taken from points a–l during field observations in spring, summer and fall of 2008. Stars (☆) represent other points or areas where water quality readings were taken. Readings at points d and l (as well as XRF data) corresponded closely to those from points further upstream. EC values are higher and pH values lower near points of drainage output (c, d), and moderated in areas where dilution by upstream water occurs (b, e, f, g, h). The lowest EC and highest pH values occurred at point l, at which water was coming from upstream areas of the Gotani River. Water quality is gradually moderated from near a drainage pipe to the area where drainage mixes with cleaner waters from upstream (i–k).

closely with readings at points a and l, respectively. DO values were relatively constant, with higher values in areas with greater water volume and turbulence. Water temperature was moderated (warmer in early spring, cooler in summer) in areas nearest points of groundwater release, with upstream areas more affected by ambient conditions. In March, at most points Eh values fell into the range expected of mine waters (roughly +0.6 – +0.8V), with upstream waters showing values in the range of those expected in rivers (+0.4 – +0.6V) and at some points near groundwater output points the values became closer to those expected of groundwater (+0.1 – +0.3V) (Baas-Becking et al., 1960; Langmuir et al., 2004). In periods with lower flow volume, all readings were within the +0.3 – +0.5 V range. In every season, all points indicated oxidizing conditions.

Optical Microscopy

Microscopic observations revealed the presence of diatoms and other microbiota both in the water and in association with the bryophytes. The presence of unique structures called oil bodies in the plant cells (Fig. 1c, single arrow) confirmed that the bryophyte is a leafy liverwort. In UV observations, many cells appeared to contain a mineral-rich fluid, with occasional points of what appears to be early mineralization (d, arrows). Chloroplasts in young cells were grouped around the cell edges (e).

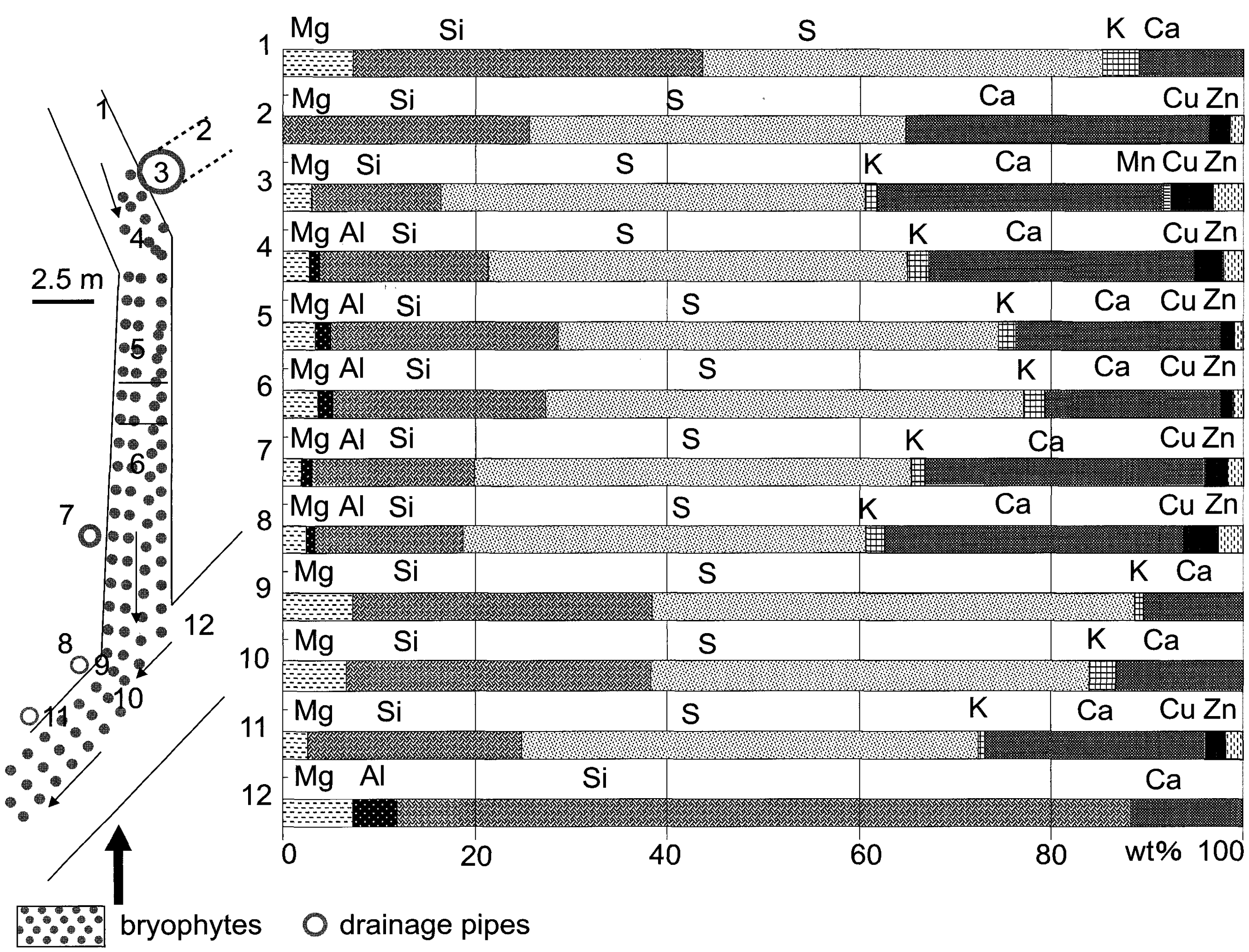

FIG. 3. Changes in metal and sulfate content by sampling point as indicated by XRF analyses of water samples. Although they were not detected upstream (1), the metals Cu and Zn were present at detectable levels in all samples from pipes and in the tributary below the first major point of discharge (2–8, 11). Based on pH levels, S is likely in the form of H_2SO_4 and all metals are likely ionized (including Al) at points of drainage output. Because of dilution effects and detection limits, most metals were not detected at points where the drainage water mixed with cleaner upstream water (9, 10). As could be expected of mine drainage, S content was high in all water samples except for that from an area of the main river upstream from the drainage (12). The Al content of this clean upstream water is thought to be a result of the presence of clay minerals.

ED-XRF

Elemental content of water varied considerably among sampling points (Fig. 3). Metals were not detected in upstream waters, but S content was still high above the point of discharge (point 1). Contents of Cu and Zn were notably higher at and near points of discharge (points 2, 3, 7, 8, 11). Levels of Cu and Zn were moderated with distance downstream (4–6) while they were not detected in the river, where relatively heavy dilution occurred (9–10). In upstream waters of the Gotani river, only Mg, Al, Si and Ca were detected (point 12). XRF analyses (semi-quantitative data) also indicated the presence of the metal Fe with traces of Cu and Zn in all liverwort and sediment samples, and the presence of Cu and Zn but not Fe in a majority of the water samples (Fig. 4). Al and Si were detected in all samples in contaminated areas. Separate analyses of the < 2 μm fraction of sediments revealed trace amounts of the metals As, Br, Sr and Zr along with higher levels of Fe, Cu and Zn. The highest heavy metal levels were found in the liverworts and associated clayey sediments, with liverworts having the highest Cu concentrations.

XRD

X-ray powder diffraction analyses of the < 2 μm fraction of the river sediments from the point of discharge (Fig. 5) revealed the presence of not only quartz (4.27Å, 3.36Å, 2.46 Å), feldspar minerals (3.79Å, 3.20Å, 2.94Å) and chloritoid (4.50Å, 2.94Å and 2.46Å) but also clay minerals including chlorite (14.38Å, 7.16Å and 1.54Å), and mica minerals (10.09Å, 5.01Å, 4.50Å) and secondary minerals melanterite (5.01Å, 3.79Å, 3.25Å), chalcanthite (4.75Å, 3.97Å, 3.69Å) and despujolsite (4.27Å, 3.69Å and 3.36Å). After treatment with ethylene glycol (EG) peaks shifted only very slightly from 14.38Å to 15.07Å, 10.09 to 10.39Å, and 7.16Å to 7.26Å. After heat treatments peaks were smaller but showed no major shifts, appearing at 14.62Å, 10.41Å and 7.23Å. This pattern is consistent with the presence of chlorite. These results agree

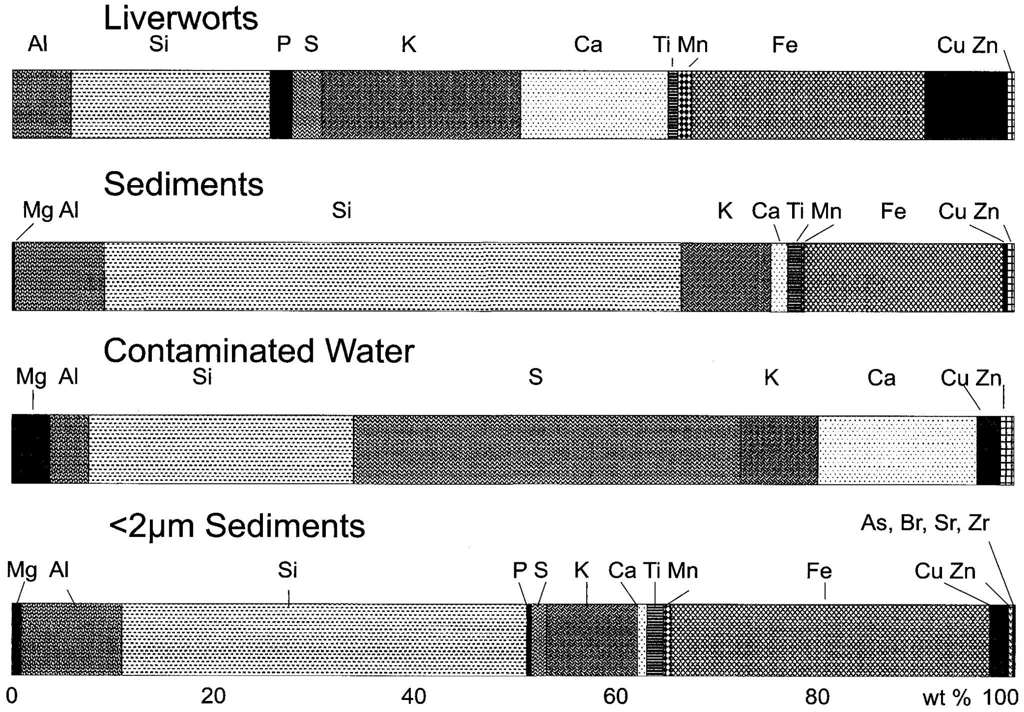

FIG. 4. Summary of XRF analyses of liverworts, sediments, contaminated water, and < 2 µm fractions of sediments from the field. Liverworts and sediments indicated the highest Fe values, while no Fe was detected in water samples. Cu content was highest in liverworts, with sediment and water samples showing reduced values or absence of the metal. Trace amounts of Zn were present in liverworts, sediments and contaminated water. Although low in the sediments, Ca content was more prominent in liverworts and in the water. Detection of Mg, Al, Si and K is consistent with the presence of mica and chlorite minerals. High Fe levels in the sediment fraction suggest the presence of Fe-oxides as well as adsorption of Fe by clay minerals. Metals such as Ti, Mn, Cu, Zn and As likely co-precipitate with Fe-oxides or are absorbed by clay minerals.

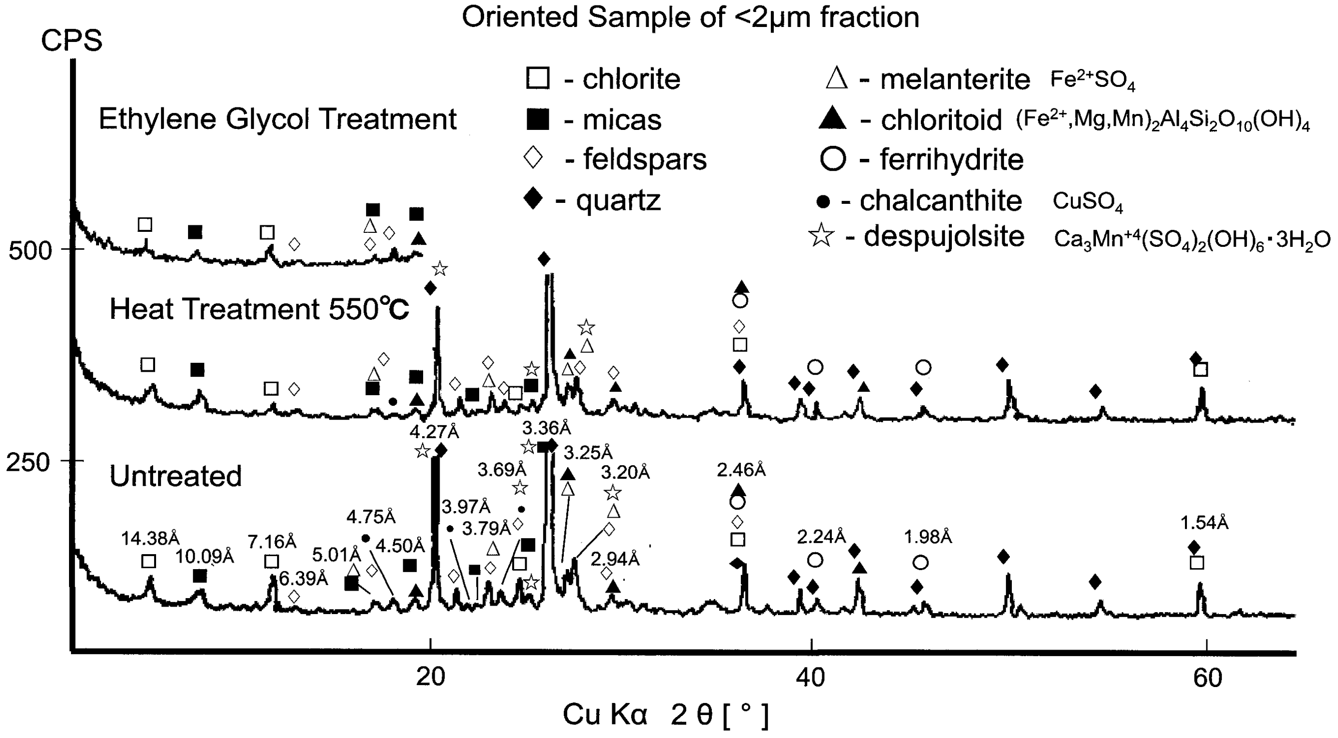

FIG. 5. Results of XRD analyses of the < 2 µm fraction of sediments from a contaminated area of the Gotani River, showing untreated, ethylene glycol treated, and heat-treated samples. The analyses revealed the presence of chlorite, mica minerals, feldspars and chloritoid ($(Fe_{2+},Mg, Mn)_2Al_4Si_2O_{10}(OH)_4$) as well as the sulfate minerals melanterite ($Fe^{2+}SO_4$), chalcanthite ($CuSO_4$) and desupjolsite ($Ca_3Mn^{+4}(SO_4)_2(OH)_6 \cdot 3H_2O$). These minerals are thought to contribute to the uptake of metals in the mine drainage environment.

well with the corresponding XRF data. It is likely that further minerals are present in trace amounts below the detection limit.

Scanning Electron Microscopy with Energy Dispersive X-ray Analysis (SEM-EDX)

Scanning Electron Microscopy showed micron and nano scale images of the sediments and abundant diatoms and other microorganisms in association with the liverworts, and

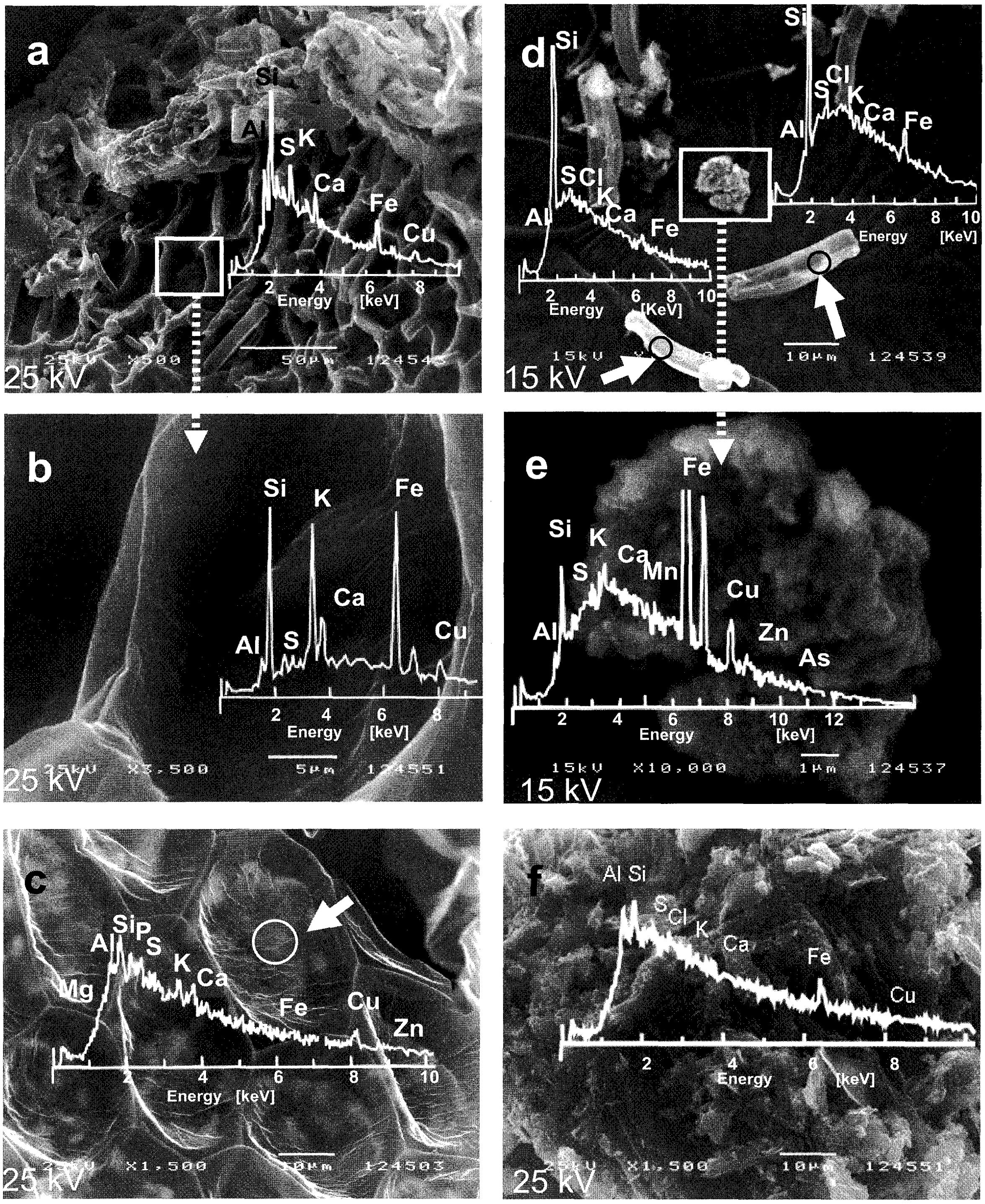

FIG. 6. SEM images and EDX analyses (area: a, b, e and f; point: c and d) of liverworts, diatoms and sediments from Gotani River. Liverworts (a–c) showed high Fe content in most instances, as well as Cu, particularly when naturally dried and covered by a clay thin film. Traces of Zn were also evident in some analyses. Diatoms (d, arrows) showed high Si associated with organic content as well as Fe. Sediments (e–f) typically contained the metals Fe and Cu, with some showing extremely high Fe content. One sediment particle thought to be an Fe-oxide (e) showed extremely high Fe content and high levels of Cu as well as traces of Zn and As. All samples showed Al and Si, suggesting the important role played by clay minerals in the ecosystem.

Energy Dispersive X-ray Analysis (EDX) provided detailed, semi-quantitative information about the location of metals within the benthic biomass and argillaceous sediments (Fig. 6). Liverworts showed consistent Cu content and variable Fe (a–c), while the elemental contents of diatoms (d) and clayey sediments (e–f) varied. In all sample types Fe was typically present, with most sediments showing extremely high Fe content (e). An Fe-oxide closely associated with a liverwort also showed Cu content as well as traces of Zn and As (e). While all liverwort analyses showed the presence of Cu, diatoms typically indicated the presence of Fe more than any other metal (d). Based on averages of all points analyzed in liverwort-containing samples (Fig. 7), related sediments contained the highest weight percent of Fe and Al, while liverworts had the highest weight percent of Cu. Contents of Zn were similarly low between sediments and liverworts, while it was undetected in diatoms. Although P, S, Cl and K were highest in the liverworts, they also were prominent in some sediments, indicating an important organic component in the particulate matter. Overall, the elements Mn and Mg were by far the highest in the liverworts, but Mn was present to a limited extent in some sediments as well. Separate analyses of bulk sediments revealed that Fe-oxides and clays are clearly the dominant minerals in the AMD environment, and there are significant S and Fe impurities associated with many of these clays and some Fe-oxides.

Transmission Electron Microscopy (TEM)/Field Emission TEM (FE-TEM)

TEM and FE-TEM observations and elemental content maps (semi-quantitative data at the micron/nano scale level) revealed that metals were associated with the bryophyte as well as with diatoms and bacteria. FE-TEM observations and elemental content mapping revealed the association of organic materials from the bryophytes with Cu. The metal was associated with the element C, seeming to bind evenly throughout the organic matter and showing concentrations in thicker areas. Furthermore, Fe, Al and K rich granules, and Al and K containing particles were also found within the cell area. A diatom, made up of SiO_2 associated with the liverwort cell showed the presence of Al, Fe, S and K within its frustule. Furthermore, some particles composed of Al and K were seen in association with bryophyte materials as well as with a diatom.

A diversity of bacteria were also observed playing a major role in metal cycling. Individual bacteria were shown to accumulate a variety of metals including Ti and Fe (Fig. 8a). Concentrations of Na, Mg, S, Cl, and Cu were fairly consistent throughout a group of three 1 μm wide and 2 μm long bacillus-type bacteria. Among the group, at least one bacterium appeared to be involved in biomineralization, and besides having the highest K content, it was coated with Si, O and associated Al and Fe. This high K level could indicate that

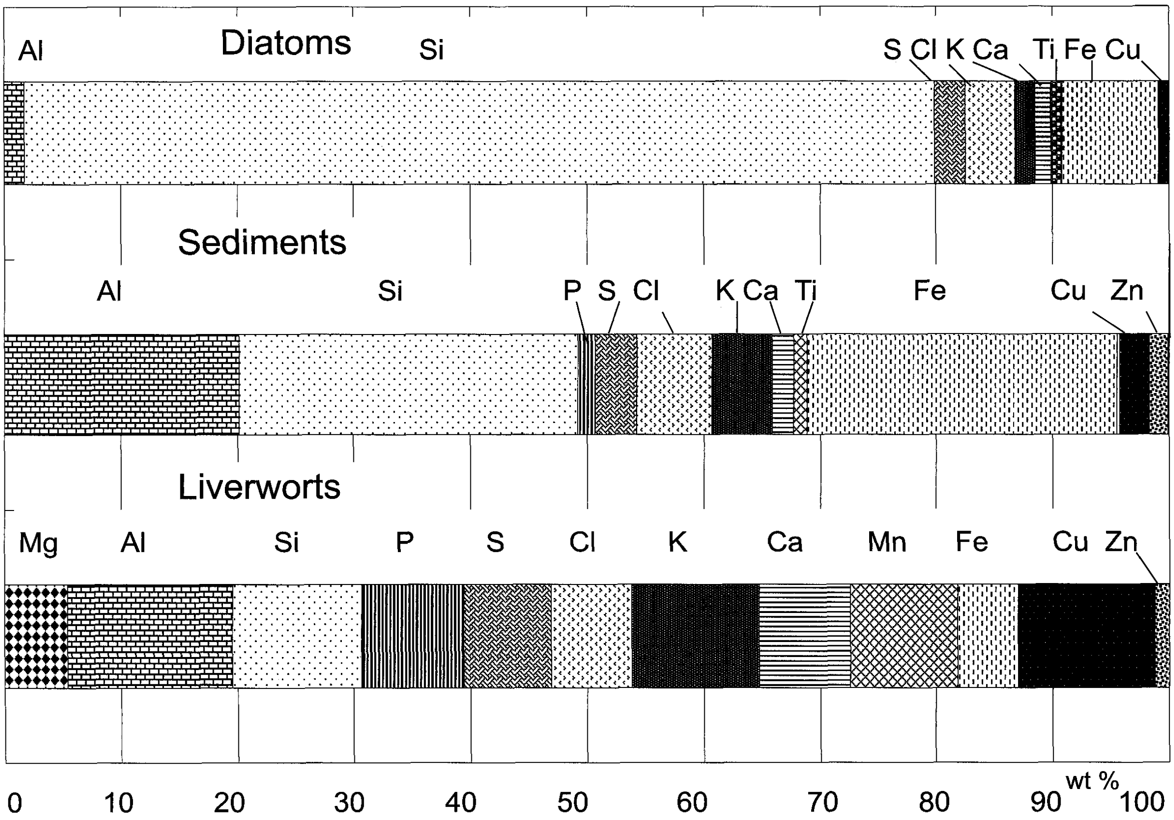

FIG. 7. Average of point analyses of diatoms, sediments and liverworts by SEM-EDX. The highest average levels of Cu were associated with liverworts, whereas average Fe content was higher in sediments and diatoms. Presence of the elements P, S, Cl and K suggest that organic content may be high overall in the sediments. The metal Zn was found in trace amounts in sediments and liverworts, but was not a significant component of diatom elemental content. High Mg content in liverworts could be associated with chlorophyll content, and Al and Si levels could suggest the adhesion of clay minerals.

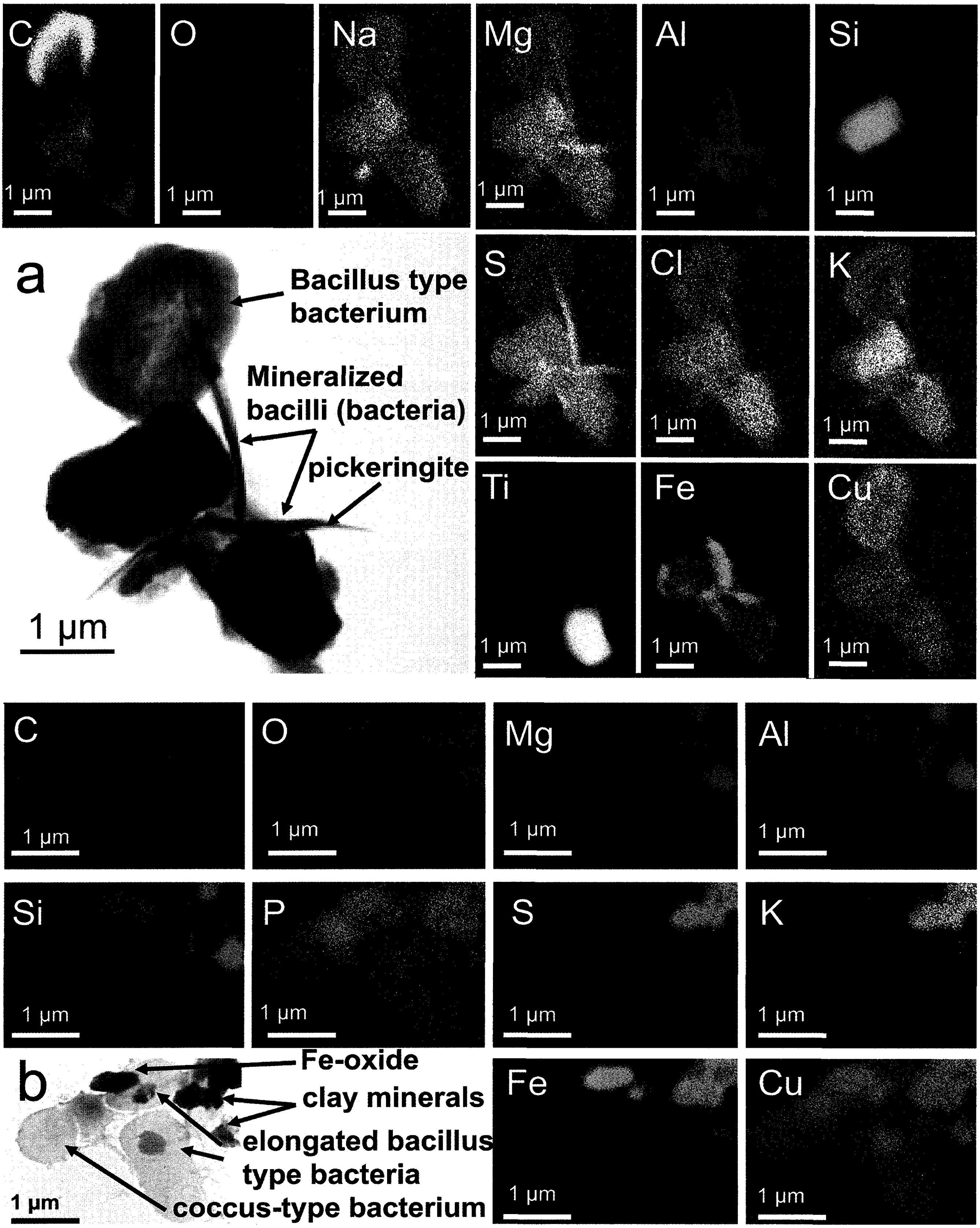

FIG. 8. FE-TEM-micrograph and elemental content maps of a group of 1 μm wide and 2 μm long bacilli from the mine drainage (a) and diverse bacteria associated with minerals (b). Concentrations of Na, Mg, S, Cl, and Cu are fairly consistent among the bacteria (in a), while each individual displays unique elemental content. Two bacteria appear to be mineralized, one with Ti and traces of Fe, and the other with Si, O and Fe. These bacteria are associated with what could possibly be fibrous pickeringite ($MgAl_2(SO_4)_4 \cdot 22H_2O$). The elongated bacillus bacterium (in b) could possibly be *Theobacillus ferooxidans* or *thiooxidans*, which are abundant in mine drainage environments. Concentrations of S, K, Fe and Cu appear with sediments and what appear to be bacterial metabolites in (b).

the mineral coating provides some protection against acidic metal-laden waters. The group was associated with what appeared to be a mineral of the halotrichite-pickingerite series ($(Fe,Mg)Al_2(SO_4)_4$), which is known to be a product of pyrite weathering. Although it is reasonable to conclude the presence of this mineral by ED-XRF data, it was not present in amounts sufficient to identify it by XRD analysis (4.82Å, 4.32Å and 3.51Å). The mineral likely formed during evaporation, possibly with the aid of these bacteria.

Other bacteria (Fig. 8b) were observed in association with Fe-containing clay minerals composed of O, Si, Mg, Al and Fe. A coccus-type bacterium 0.75 μm in width, and an elongated bacillus-type bacterium 1.5 μm in length show no signs of mineralization or metal uptake on the cell bodies, while a smaller bacillus was associated with an Fe-oxide mineral. Both bacteria and minerals contained Cu.

A coccus-type bacterium 1 μm in width was observed surrounded by minerals, and elemental content mapping revealed that it was producing Fe-oxides. Concentrations of Al, Si, P and K in and on the bacteria indicate that it had attracted clay mineral particles to its surface as well.

DISCUSSION

Ogoya's Gotani River, although tainted by AMD-associated toxins, exhibits natural remediation processes by various components of the benthic ecosystem (Fig. 9). With pH changes some metal ions are precipitated with or adsorbed by minerals (Fe>Si>S>Al>Cl>K>Ti>Cu>Zn>Ca>P>Mn>(Ni,As), while others are accumulated by diatoms (Si>Fe>Cl>S>Al>K>Ca>Ti), liverworts (Al>Cu>K>Si>P>S>Mn>Mg>Ca>Cl>>Fe>Zn) and bacteria. These processes contribute to partial amelioration of mine drainage, but not to the extent that currently allows the return of healthy fish populations and biodiversity. Clear challenges remain, and this study has helped to clarify some of the problems to be addressed. Worldwide studies have identified patterns associated with mine drainage similar to those observed in Ogoya, and the contamination in the groundwater released to this area of the Gotani river seems to clearly be a result of past mining activity.

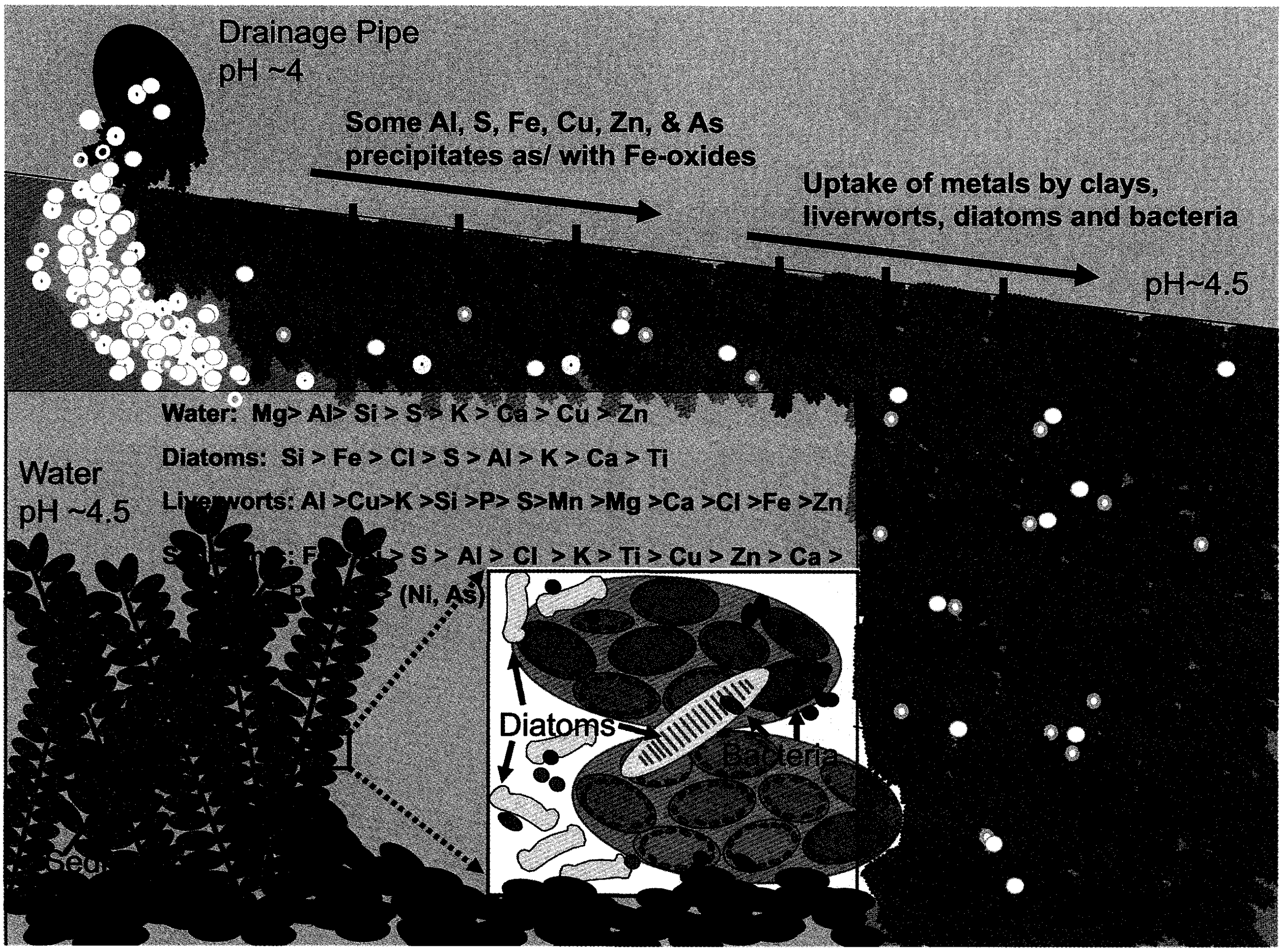

FIG. 9. Overview of the benthic ecosystem and metal cycling. Water pH increases from ~pH 4 at the drainage pipe to ~4.5 downstream through physical, chemical and biological processes. Fe-oxides rapidly precipitate from the water along with other metals including Al, S, Cu, Zn and As. Remaining metal ions (Mn, Fe, Ni, Cu, Zn, As) are adsorbed by clays, liverworts (Al, Fe, Cu, Zn), diatoms (Fe, Al, Cu, Ti) and bacteria (Al, P, S, Fe, Cu, Ti). These natural processes contribute to improved water quality downstream.

Water Quality

Water quality in rivers affected by mine drainage can vary drastically. In some cases the pH is extremely low, such as the Rio Tinto in Spain with a pH of 1.5–3.1, or the Richmond Mine in California pH 0.5–1.0 (Bond et al., 2000; Ashley et al., 2003; Gonzalez-Toril et al., 2003; Baker et al., 2004). At other sites mine drainage waters are less acidic or even neutral but retain other AMD characteristics such as high S and metal levels (Scharer et al., 2000; Gerhart et al., 2005; Gomes and Favas, 2006). Studies in Tennessee, and North Carolina, USA, revealed that some streams located in the watersheds of abandoned Cu mines actually had higher pH than other nearby streams unaffected by mining but draining areas with exposed sulfur minerals (Hammarstrom et al., 2003). Compared with many of these locations, Gotani River's pollution is relatively mild, but even so the environmental impacts have been severe, and residents have suffered health consequences.

A comparison between EC values and metal content of water from the drainage ditch and the river revealed a clear influence by the metals on the electrical conductivity. The metals Cu and Zn were detected in many water samples, and these elements are among the most harmful for the environment (Kabata-Pendias and Pendias, 2001). High ionized metal content is expected based on XRF results of water samples and the pH and Eh values on site, and it is assumed that these metals are highly bioavailable in the drainage water from Ogoya mine (Drever, 1997; Langmuir et al., 2004). This contributes to their toxicity to aquatic organisms, particularly in the case of Al, Cu and Zn (Allen et al., 1980). The presence of Ca in the water, however, likely helps to moderate the negative effects of the mine drainage for organisms in the ecosystem, although the Ca levels are not particularly high. Detection of Mg, Al, Si and K is consistent with the presence of waterborne clay minerals (mica and chlorite).

Water from the large drainage pipe showed pH range of 4.0 to 4.5, with the lowest pH reading being made in March, coinciding with snow meltoff. This could be expected as winters in the area feature more snow than rain, and thus there must have been considerable buildup of secondary minerals, such as melanterite and chalcanthite, within the mine (Hammarstrom et al., 2003). With rapid snow meltoff and March rainwater penetrating into the mine, these highly soluble minerals would rapidly dissolve, creating particularly acidic, metal and sulfur-laden drainage.

Dissolved oxygen (DO) during heavy flow in March ranged from 7.3–10.9 mg/l, which is enough to sustain fish and plants. However, in low-flow seasons it was 1.5–3.5 mg/l, (saturation 11–30%) with mostly around 2.5 mg/l, likely influenced by Fe-oxide precipitation. Fish cannot survive these DO levels.

Sulfate is the most abundant contaminant of the river in this study, and is a defining characteristic of AMD in general. In fact, on a global basis around 150 million tons of sulfate in rivers and oceans can be traced to mining and AMD (Brimblecombe et al., 1989; Edwards et al., 2000). The persistence of high S levels even when metal levels become undetectable (by XRF) coincides with previous observations of AMD affected rivers, and is explained by ionic character (Nordstrom and Ball, 1985; Gomes and Favas, 2006)

Although the water quality in Gotani stream limits biodiversity and is inhospitable to fish, its chemistry is still superior to many mine-drainage affected streams worldwide. With dilution by clean upstream waters, pH rapidly returns to neutral in parts of the stream, and toxicity of many metal ions is ameliorated through resulting precipitation, dilution, adsorption and other natural processes. This increases hope that future enhanced remediation efforts could bring back healthy natural ecosystems capable of supporting fish.

Sediments

A geochemical mapping of the Hokuriku region indicated abnormally high Cu, Cd and Pb levels in sediments collected in the watershed of Ogoya mine, thus although Cd and Pb were not detected in the current study they are possibly present seasonally or in levels below the detection limits of the equipment used (Ohta et al., 2004). Throughout the study area, the drainage ditch was completely covered in concrete, limiting the sediment load, and although it was shallow, water flow was fairly rapid.

The majority of sediments collected were those associated with benthic bryophytes, which provide shelter against the current and prevent some particulate matter from being swept away. Clays and Fe-oxides made up a major portion of all sediment samples in contaminated areas, which corroborates well with XRD results. While no Fe was detected in water samples, it was present in large amounts in sediments, which is consistent with the rapid precipitation of Fe-oxides, which can co-precipitate trace metals such as As through processes including bacterial mediation and photooxidation (Bednar et al., 2005). Such an As-containing Fe-oxide was observed by SEM, and these minerals reduce the bioavailability of As, with beneficial effects for the surrounding benthic organisms. Previous studies showed that minerals including calcite, gypsum, ettringite and smectite can fix metals such as Fe, Mn, Cu, Cd and Zn, and this phenomenon was observed in a different drainage area of the Ogoya mine treated by lime (Veiser, 1983; Sato and Tazaki, 2000, 2001). SEM-EDX content maps from a study by Sato and Tazaki (2000) showed that of the minerals in that area, calcite had the highest overall concentrations of heavy metals and accumulated large amounts of S and traces of Fe, Cu and Zn; while gypsum and ettringite accumulated only traces of Fe, Cu and Zn. These three Ca-rich minerals were not prominent in the relatively Ca- poor, untreated area in the current study, although Fe, Cu and Zn were associated with clay sediments, precipitates and traces of secondary minerals. Despujolsite, a mineral with a structure similar to ettringite, was identified in XRD analyses, and it may play a similar role in metal uptake at the study site (Zhou et al., 2004).

Secondary sulfate minerals can be major contributors to acidity, and can result in lowered pH near naturally weathering rocks (Bond et al., 2000; Hammarstrom et al., 2003). They result from weathering of sulfide minerals, and acidity and metal ions are a by-product of the weathering reactions (Bond et al., 2000; Hammarstrom et al., 2003). These highly soluble minerals are an important sink of metals, but in the event of exposure to water, as in a heavy rain, they can create rapid bursts of acidity and metal contamination in runoff (ibid.). Similar events within the Ogoya mine and to a small extent along the river banks are likely one factor preventing the return of healthy fish populations to large stretches of Gotani river. Although detected by XRD, their ephemeral nature and rapid disintegration prevented their unequivocal identification by SEM. However, even a small amount of these minerals' presence is a concern.

The presence of sulfate minerals such as jarosite (as well as melanterite) is common in mines, and they are targets of microbial action and sources of potential acidity through simple dissolution (Bond et al., 2000; Hammarstrom et al., 2003) As in this study, secondary minerals including chalcanthite were previously found in association with stream sediments in an AMD environment in Australia (Ashley et al., 2003). Chalcanthite, a copper sulfate, is poisonous, and some forms of copper sulfate are used to control algae, fungi, etc. (Schlenk and Moore, 1994; Chen and Lin, 2001). FE-TEM observations also showed what could be pickeringite associated with bacteria. The mineral was not identified by XRD in the < 2 μm fraction of sediments, but that is to be expected of this and other highly soluble minerals which tend to be present only in small amounts in bulk mine drainage sediments (Dold, 2003). Although these minerals are highly soluble, they are less likely to dissolve in the presence of elevated levels of ions of their constituent elements, and in this case, sulfates (Manahan, 2005). Heavy metals are known to be removed from aqueous media through the re-formation of minerals (Bigham et al., 1996). In a marine hydrothermal vent environment, high levels of sulfide have been correlated to lowered toxicity of Cu and Zn because of "the formation of dissolved metal-sulfide complexes" and a resulting decrease in bioavailability (Edgcomb et al., 2004). Similar phenomena are at work in Ogoya, lessening the toxicity of metals for certain organisms, including bacteria. The prominence of metal sulfates' role in metal toxicity is weather related, but they must be considered in any remediation plan for Ogoya even when present only in trace amounts.

In studies of tailings from the Witwatersrand Goldfields in South Africa, chloritoid was shown to be the predominant silicate in tailings sediments, present in greater quantities than even quartz (Cukrowska et al., 2004). In fact, it has often appeared in association with quartz inclusions (Halferdahl, 1961; Cukrowska et al., 2004). The mineral has been found worldwide, often in association with schists, quartz veins, hydrothermal veins and faults, and it appeared to have been formed along with chlorite and pyrite at a site in South Africa (Halferdahl, 1961). It is often found with quartz, muscovite mica, chlorite and iron oxides (ibid.), which were also present in the sediments analyzed in this study. Replacements of Mn or Mg for Fe(II), and of Fe(III) for Al are common in chloritoid, which has the basic formula $H_2FeAl_2\ SiO_7$ (Halferdahl, 1961). Thus, a portion of the Fe in sediments from Gotani River may be attributed to chloritoid.

High Fe levels in the sediment fraction also suggest the presence of Fe-oxides as well as adsorption of Fe by clay minerals, and this is corroborated by XRD and SEM-EDX data. Metals such as Ti, Mn, Cu, Zn and As likely co-precipitated with Fe-oxides or are absorbed by clay minerals, particularly at pH > 4 (Ashley et al., 2003). Sulfate is also able to adsorb to clay minerals and "Fe and Al hydrous oxides," particularly at the pH levels found in this study (Pierzynski et al., 2005). Indeed most clays were found by SEM-EDX to be highly enriched in Fe and sulfate, and Fe oxides also tended to show high S contents. Depending on pH and the ocre type, different metals are more or less likely to adhere to precipitated minerals in AMD (Dzombak and Morel, 1990; Webster et al., 1998; Hammarstrom et al., 2003). With pH increases, metals tend to precipitate or sorb to minerals in a designated order, with Cu typically preceding Zn (Dzombak and Morel, 1990; Munk et al., 2002). Studies show that Zn and Mn do not sorb to ferrihydrite until pH approaches neutral, and thus they may be more persistant contaminants in AMD and present a larger concern in Ogoya than Cu (Dzombak and Morel, 1990). This also explains the rarity of Zn in sediments in this study. Goethite more readily takes up metals at lower pH, however it was not identified in this study (Hammarstrom et al., 2003). Also, high SO_4^{2-} levels may facilitate sorption (Munk et al., 2002). Microenvironments in small areas among liverworts may influence sorption through such effects.

It is clear that minerals, and particularly clay minerals, play a crucial role in the cycling of metals in Ogoya mine and the AMD-affected ecosystem in Gotani stream. The presence of exposed sulfide minerals in the mine leads to drainage enriched in metals and S. Adsorption of metals by sediments and particularly clays extracts metal ions from the water, decreasing their bioavailability and thus improving the water quality for benthic organisms. With changes in pH and DO, certain elements combine to form minerals which precipitate out of solution, further improving water quality.

Biological Processes: Bryophytes, Diatoms and Bacteria

Previous studies have indicated that bryophytes in rivers often reflect their geological setting even more accurately than the surrounding water or sediments (Samecka-Cymerman and Kempers, 1998). Indeed, the contents of Cu and Zn were consistent overall in the liverworts, while Fe was variable; in bulk sediments there was larger variability in metal content; and water contained relatively low concentrations of all metals. Clay fractions of sediments, however, contained the highest levels of metals, and particularly Fe.

Analyses of all parts of naturally dried liverwort samples showed consistently high Fe levels, while those of freeze-dried liverwort samples showed consistent Cu levels but dramatically decreased Si, Fe and sometimes Al levels. This could indicate that Fe is accumulated primarily in the exchangeable fraction on the bryophyte's surface, likely through precipitation processes or adsorption onto a thin clay layer; loosely attached elements and clay minerals may be displaced during repeated rinsing and centrifugation with ethanol and T-butyl alcohol in the freeze-drying process. The consistency of Cu and Zn levels across all sample types suggests that their uptake is internal, and thus they are not displaced. Liverworts' sheltering of clays and Fe-oxides from currents encourages metal accumulation by these minerals and limits their mobility.

It is not clear exactly what mechanisms give the bryophyte its tolerance to the AMD conditions. Oil bodies, structures that are unique to liverworts, contain dissolved proteins, terpenes and enzymes, and produce essential oils which have various properties, some of which may aid in metabolism, or play protective roles (Pihakasi, 1968; Asakawa, 1998; Suire et al., 2000; Heinrichs, 2003; Martins Adio, 2005). One negative aspect of these terpenoids may be toxicity to certain bacteria which help to precipitate minerals using ionic metals and S from the AMD solution (Neculita et al., 2008). Thus, although the liverwort provides shelter and adsorption sites, and removes Cu from solution, it may negatively impact some part of the microbial community, decreasing mineral precipitation. Indeed bacteria were not abundant in areas covered by liverworts, while microbial mats were present in other parts of the riverbed.

In some areas the liverwort is present in volumes greater than or equal to the flowing water, particularly in dry periods. It is possible that photosynthetic processes by the liverwort and other benthic organisms contribute to the moderation of pH in this system, influencing the pH values (between 4–5 in most bryophyte covered areas) below the initial point of discharge.

Although they are composed of predominantly Si, Fe is also essential to diatoms, and studies have shown that some diatoms' surface ligands facilitate its uptake by converting Fe(III) from the aqueous environment to Fe(II) and binding it (Shaked et al., 2005). Although the study focused on marine diatoms, this may be an important process in mine drainage waters as well. Diatoms observed in this study frequently showed Fe uptake which agrees with the results of previous studies (Chen et al., 2003; Nakanishi et al., 2004)). Chen et al. (2003) suggest that Fe uptake by diatoms is facilitated by its prior binding or complexation. It has also been observed that Cu and Zn tolerance is more often found in diatoms that are able to live in acid environments (Haslam, 1990). This tolerance may be related to the diatoms' metal-binding capacity. Diatoms benefit by attaching to liverworts for stability and accumulating Fe and S.

An abundance of bacterial species have been identified as contributing to the metal cycle and production of AMD (Bond et al., 2000; González-Toril et al., 2003; Baker et al., 2004; Enders et al., 2006; Hallberg et al., 2006; Edwards et al., 2008). According to Lazaroff (1963) and Brady et al. (1986), the influence of sulfate [in AMD] is important because in order to carry out the reactions that create iron containing precipitates, bacteria that convert Fe(II) to Fe(III) require the presence of sulfate (Lazaroff, 1963; Brady et al., 1986). With its high sulfate content, Ogoya's AMD is thus a promising environment for their action. Such bacteria were observed precipitating Fe oxides by FE-TEM observation (not pictured), removing the limited bioavailable Fe from solution and possibly co-precipitating traces of Al, S and Cu.

Furthermore, although metal and sulfur oxidization is most often associated with low pH conditions inside of mines, studies have identified bacteria which are active, even in alkaline conditions, in the oxidation of Cu and S containing minerals and subsequent metal release (Simpson et al., 2005; Church et al., 2007). Previous studies have shown that sediments can be a source of toxic metals even outside of AMD impacted areas (Ashley et al., 2003). This implies that although pH is ameliorated downstream by dilution and other processes, bacterial oxidation can still contribute to increased levels of toxic metals all along Gotani River, as long as AMD-related sediments and precipitates, particularly clay minerals, continue to be washed far downstream.

On the other hand, many studies have shown bacteria's ability to adsorb, mineralize and otherwise take up contaminants including heavy metals from the environment. According to Tazaki (2000), accumulations of Fe and Mn adsorbed to bacterial cell walls can be converted to biominerals through the addition of OH- groups. Kishigami et al. (1999) reported that Cu was captured in and on bacterial cells near the Ogoya mine. These mechanisms may allow metal uptake and subsequent biomineralization in this environment as well. Other bacteria create outer coatings made of Si, through "nucleation, polymerization and adhesion of silica ion[s] and/or colloidal silica," (Asada and Tazaki, 1999). Such mechanisms have been observed in acidic and high sulfur environments (Asada and Tazaki, 1999, 2001). Such a silica coating could be a protective mechanism. A few Si spherules very similar to those observed previously were found in AMD samples, associated with a liverwort (not pictured). According to Urrutia Mera and Beveridge (1993), such bacteria can sometimes create surficial "metallic silicates," particularly in natural aquatic systems high in Si, Al and Fe, much like the conditions in Ogoya. This is consistent with a Si-coated bacterium observed by SEM (not pictured) and the Fe-enriched areas of the Si-coating observed on a bacterium in Fig.6a. This type of biotic action is crucial to the removal of toxic metals from mine drainage, and limiting the water's toxicity to benthic organisms.

CONCLUSION

In accordance with similar studies of bryophytes, the liverworts in the Gotani stream contained high heavy metal contents (especially Fe and Cu), often even higher than those in the surrounding water or sediments. Liverworts seemed to be the primary sink for Cu^{2+} ions in the water, and Fe(III) ions tended to precipitate as Fe-oxides or be adsorbed by bryophytes, clays or diatoms. Argillaceous sediments, particularly clay minerals, apparently adsorbed Cu, Zn and other trace metals to some extent as well. Another prominent contaminant, S, did not appear to be largely remediated by the benthic organisms but was prominent in association with clay minerals and, to a lesser extent, Fe-oxides. Water content varied from point to point, but typically aquatic metal ratios were lower than those in the liverworts or sediments, indicating metal accumulation at binding sites of plant and microbial cell walls and mineral surfaces. Future studies will include sequential extractions of sediments to determine their precise ratios and relative metal contents, in-depth analyses of metal uptake and mineralization processes in benthic organisms and minerals present in varying conditions in nearby areas of the Gotani River. Clearly each component of the system has an impact on the others, and metals and S are present in abnormally high levels in the water, sediments and biomass of the area impacted by drainage from the abandoned Ogoya copper mine. Remediation methods will need to take into account not only ways to enhance metal uptake by biological means, but also mineralogical processes that could be initiated based on changes in the environment and methods to avoid recontamination by metal-enriched sediments.

ACKNOWLEDGEMENTS

Our sincere thanks go out to the volunteer group from Ogoya for willing support and collaboration, and to the members of Dr. Tazaki's working group for technical support and advice. I would also like to express my appreciation to Mr. Nagatake at JEOL for his expertise in carrying out the FE-TEM analyses. Special thanks to the reviewer and Editor-in Chief Prof. E. Narita for the comments and suggestions which greatly improved this paper.

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