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A Biomineralization of Diatom in Acidic Stream Sediments

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Abstract: SEM- EDX analysis of a biomat in acidic stream sediments, Homan area, Matsue, has revealed iron mineral formation on the surface of cell wall of diatoms. Induced mineralization is biologically controlled by the water chemistry. The chemical composition of the acidic stream influence the environment of the diatoms in the biomineralization process. The chemical compositions of the thin films on the external cell wall have been changed by the ratios of Si/Fe.

1. Introduction

Iron and manganese are extremely abundant in acidic stream sediments. Both metals are distributed in many diverse environments. The metal precipitating bacteria is known to occur in sulfur contaminated lakes (FERRIS, *et al*., 1987) and in iron-rich streams (McKNIGHT, *et al*., 1988). The bacterial activity has been studied in mining area showing high pH. Special techniques to recover polluted areas has been found (GHIORSE, 1986; METAL MINING AGENCY OF JAPAN RESEARCH GROUP, 1992).

The Homan area, southeastern Matsue city, is an old copper mine which was operated from 1855 to 1865 producing copper minerals and cement copper. The geological setting is characterized by rhyolite lava and breccia layers, and chalcopyrite-pyrite-quartz assemblage in veins (INOUE, et al., 1982; TOYAO, 1985; YONASHIRO, 1985). Through the mining drainage system, the polluted water and non-consolidated sediments are continuously transported by the underground water into the streams. These sediments associating with biomats show variaties of the color in which orange, brownish-red and red are principal. These materials show a characteristic of the seasonal occurence. During the spring, summer and autumn the quantity of non-consolidated material and the distribution of biomat are maximum and the occurence subsides during the winter.

In the present paper, these materials (non-consolidated sediments with biomat) and the water were collected. The biomineralization of Si-Fe components from diatoms in the biomat were observed. The literature contains few examples of corresponding studies from the diatom biomineralization.

2. Materials and Methods

At field, the pH was measured in water using the Castany ACT pH mater D-12 (Horida). Approximately 10 ml of formaldehyde solution (Formaline 37%) was added to the bottle with collected samples. Through this procedure the organic structures were preserved. At the laboratory, the smear slides with cover glass and the thin sections were observed under the optical microscope. For the thin section the Cyanobond was used to adhere the dried material to the slide glass. XRD of the dried samples was performed using the X-ray powder diffractometer RIGAKU (RAD-C) with condition of 30 kV and 10

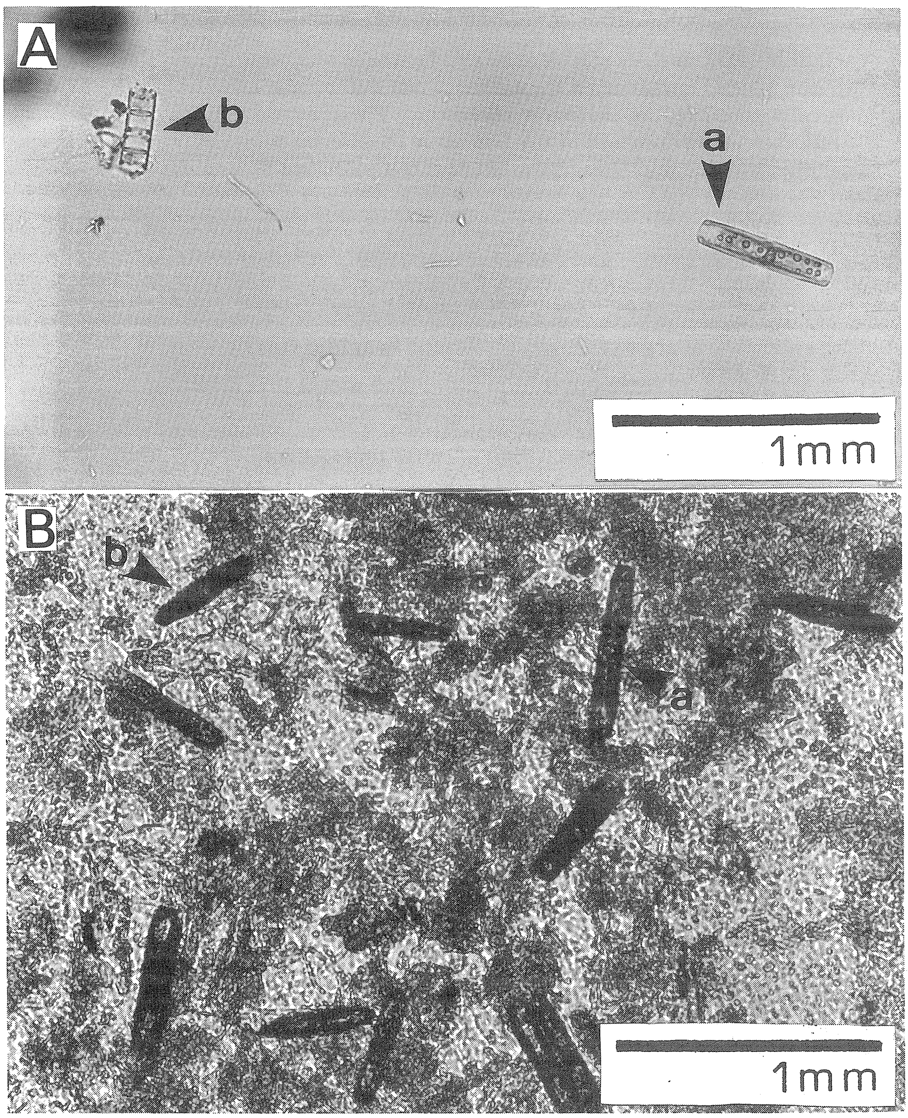

Fig. 1 Optical micrographs of (a) *Navicula oblonga* and (b) *Aulacoseira sp*. in suspended material A and (a) *Navicula oblonga* and (b) *Craticula Cuspitada* associated with the brownish-red color sediment in thin-sectioned sample B.

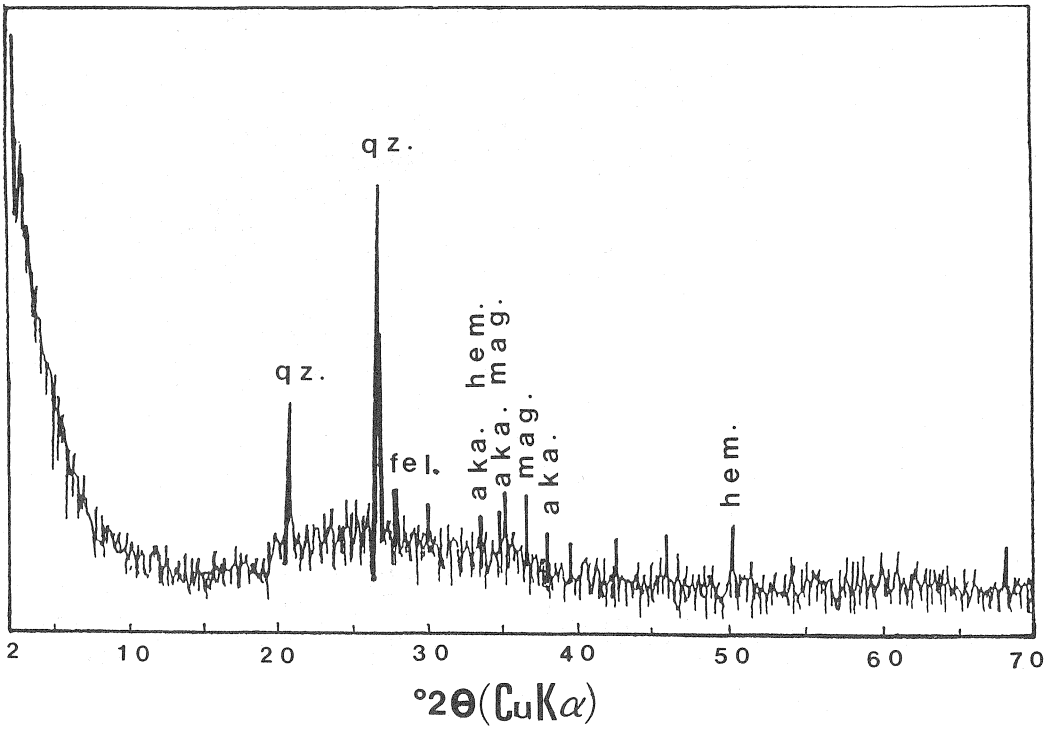

Fig. 2 The XRD results from the sample where the diatoms were found. qz.; quartz, fel.; feldspar, hem.; hematite, aka.; akaganeite, mag.; magnetite.

mA.

For the SEM and EDX analyses the materials were washed by the ethanol solution (20%, 40%, 60%, 80% and 100% concentration respectively). The material was coated with gold. The scanning electron microscope (JEOL), and the energy dispersive X-ray analysis (EDX) was done using the scanning electron microscope S-2100 (Hitachi) equipped with X-ray microanalyzer (Horiba) EMAX-3000. According to the SEM and EDX results, the most representative occurences were chosen to make the Radiofrequency Inductively Coupled Plasma (ICP) analysis of the fresh water samples. For this analysis the Multi-Spectrometer ICPS-2000 was used. A standard solution was prepared using the following elements: Fe (10 ml); Al (10 ml); Cu (5 ml); Ni (2.5 ml); Cr (5 ml); Mn (5 ml); Ti (1 ml); S (50 ml from solution of (NH_4) $2SO_2$, 4,1208 g in the water) for parameter and ionized water until a complete 1000 ml solution was formed.

3. Results

3.1 pH measurement

The pH in the streams, lakes and river in the Homan area were taken in 72 places from

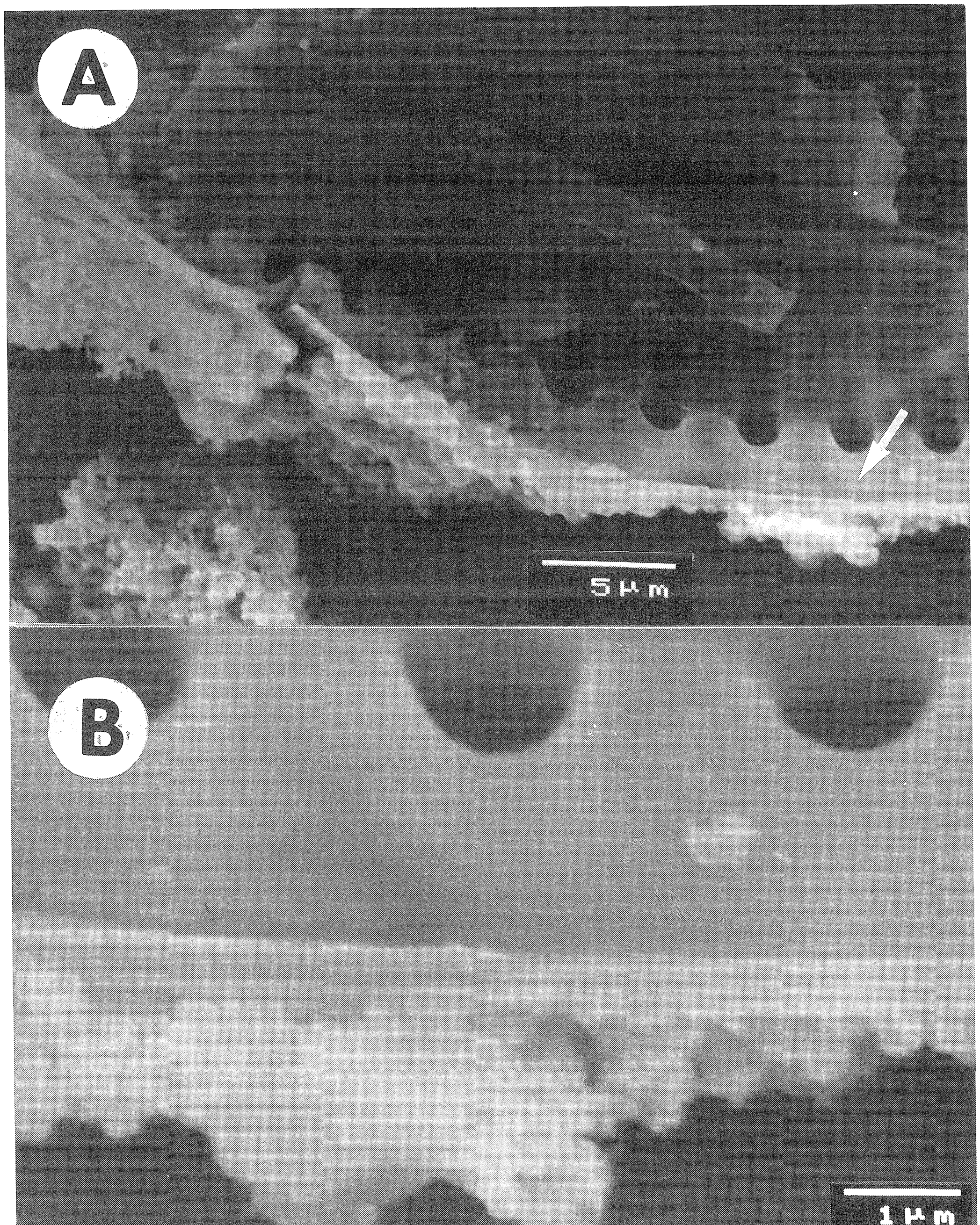

Fig. 3 SEM micrographs of a sectioned diatom valve (A). With the higher magnification (B), the film bound in the outer part of the valve is clearly observed.

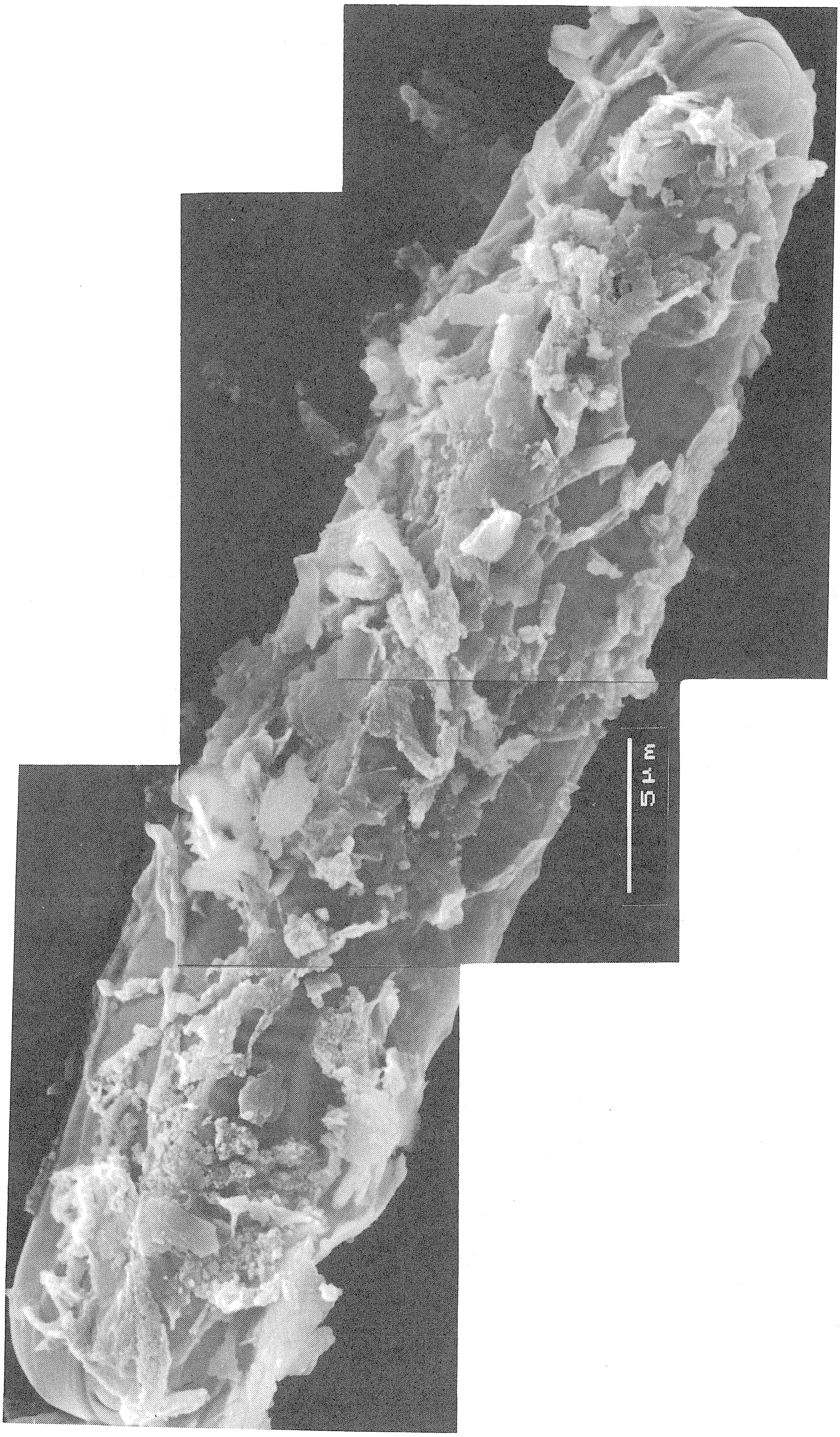

Fig. 4 SEM micrograph of *Navicula oblonga* covered with mineralic materials.

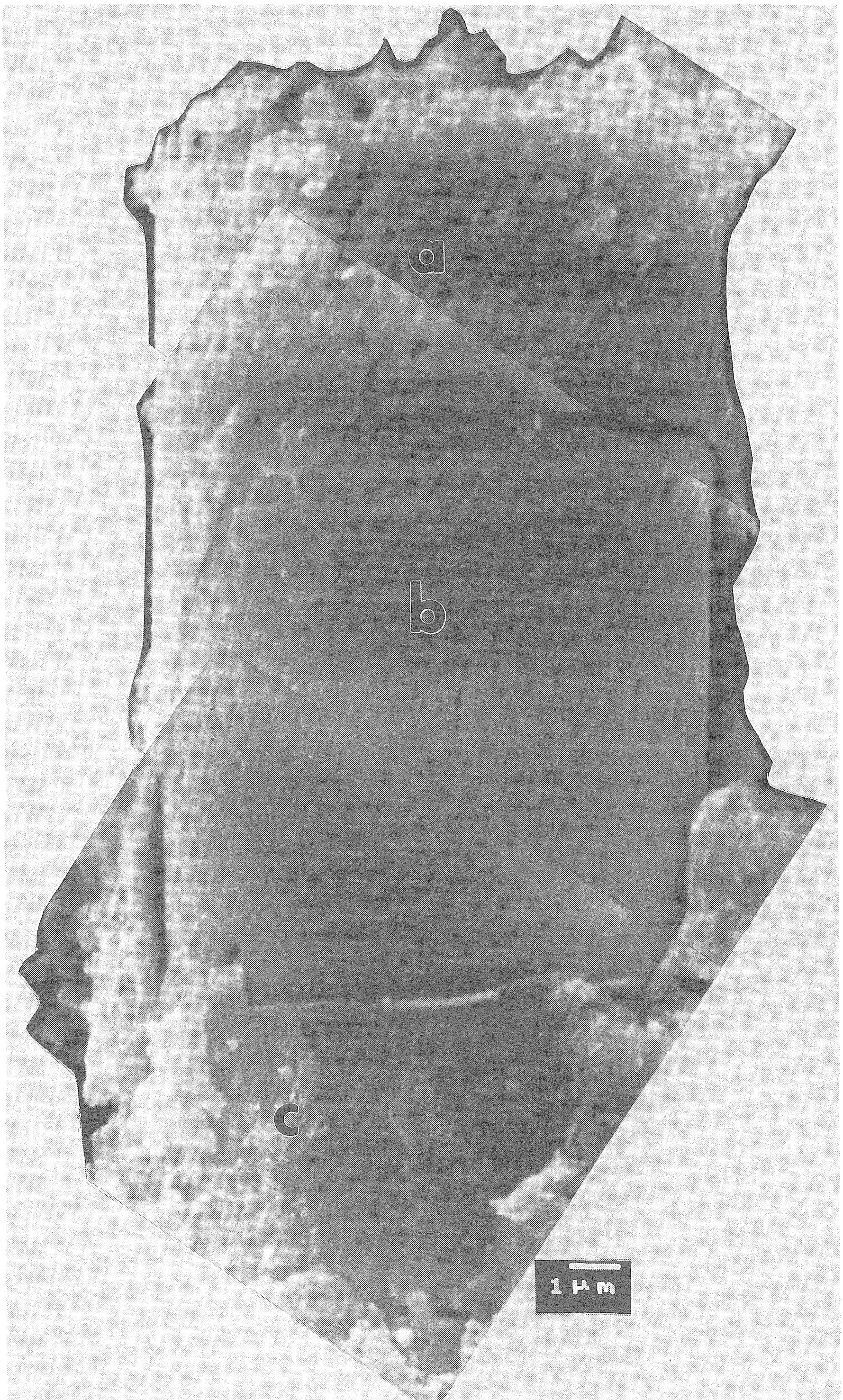

Fig. 5 SEM micrograph of *Aulacoseira sp*. covered with mineralic films. (a) the own diatom valve, (b) the smooth film and (c) the aggregate film.

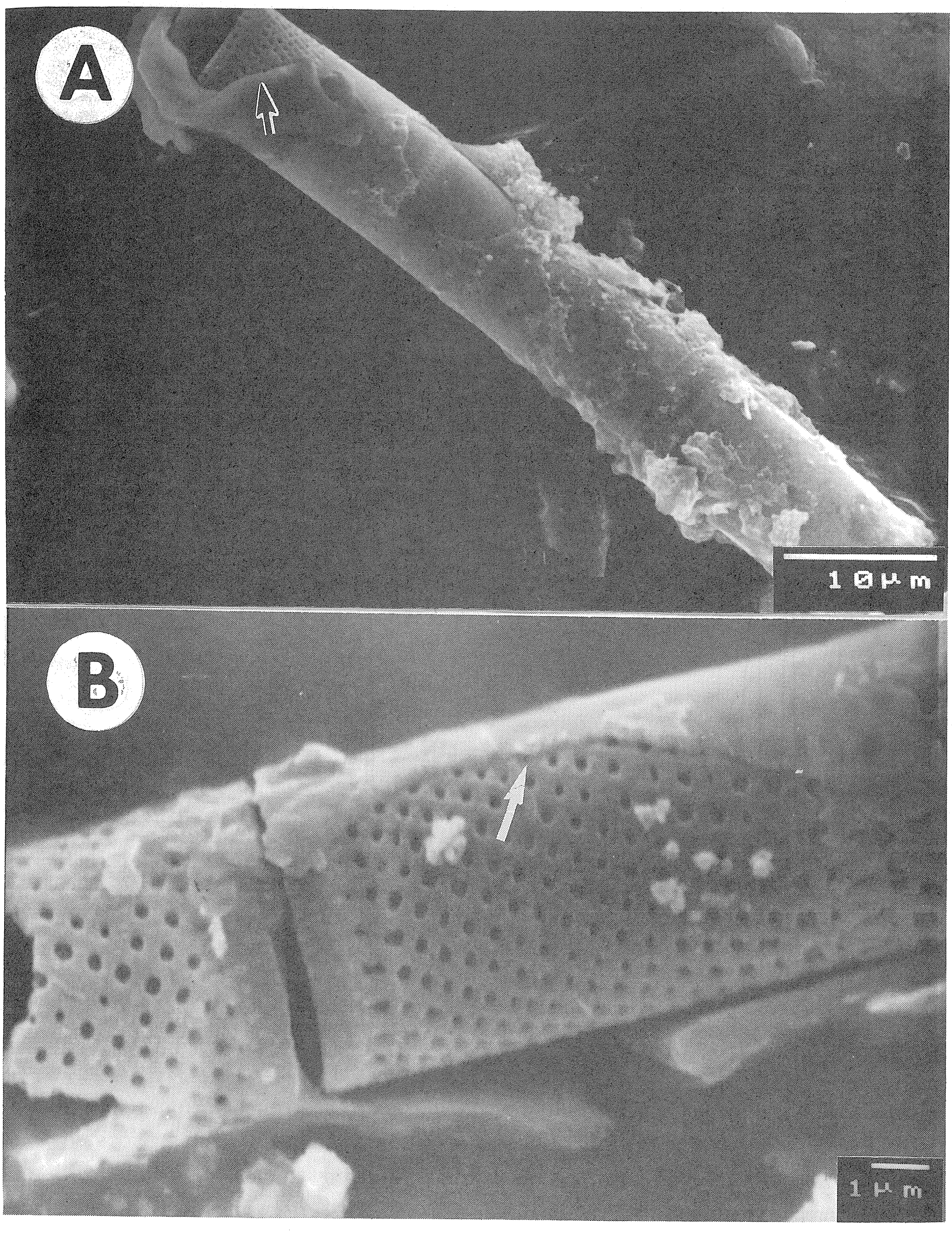

Fig. 6 SEM micrographs showing *Aulacoseira sp*. binding material in the outer part of the valve (A), (B) shows in more detail the thin mineralic film covering the valve.

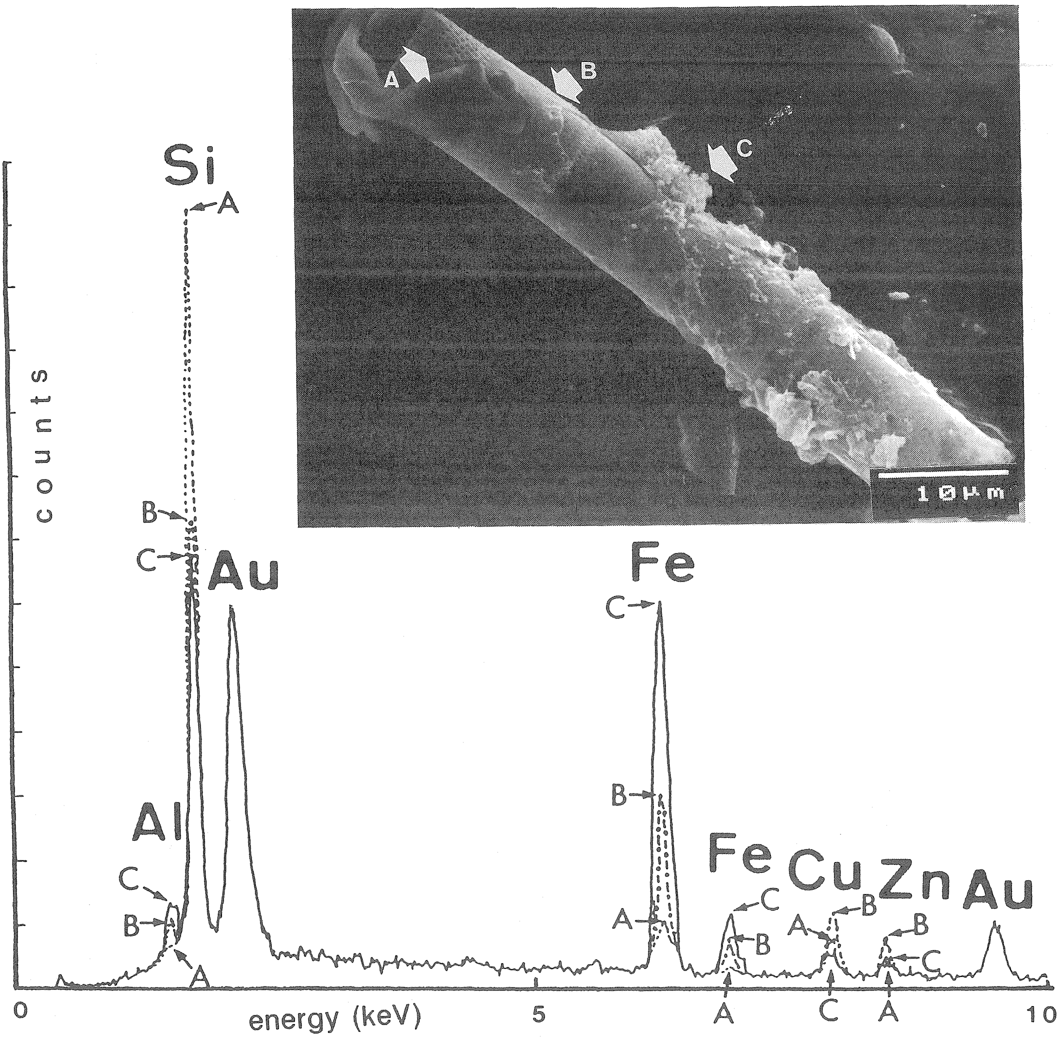

Fig. 7 SEM-EDX of *Aulacoseira sp*. showing three different compositional steps. The EDX data indicate Si, Fe and Al deposition of the diatoms'valve (A), a thin film which covers the valve (B) and the minerals' aggregates (C).

November '91 to June '92. The results revealed acidic to neutral conditions, from pH 2.9 to pH 7.4. Frequently, the low pH results were obtained in places which are located near the mining entrances, ponds and where the non-consolidated sediments associated with the biomat. In the streams the average pH was around 4.5 whereas specifically in the place where the diatoms were found, near pond, the average pH was around 3.6.

3.2 Optical microscopic observation

The optical microscopic observations revealed biomat's constituent, showing filamentous structures (algae), green-algae, twisted algae and diatoms. The diatom species were identified as *Aulacoseira sp*. (b) and *Navicula oblonga* (a) (Fig. 1A). In the thin sections, the diatoms were commonly found in brownish-red color biomat (Fig. 1B). The diatoms were identified as *Navicula oblonga* (a) and *Craticula cuspitada* (b) in the Fig. 1B, respectively.

3.3 XRD analysis

XRD of the biomat with diatoms shows a mineral assemblage composed of quartz, feldspar and amorphous Si (considering the background), associated with hematite (Fe_2O_3) with weak intensity peak at 1.84 Å, magnetite (Fe_3O_4) with weak intensity peak at 1.62 Å, and akaganeite (β-FeO(OH)) (Fig. 2). Iron minerals showing low crystallinity were mixtured in the biomat.

3.4 SEM-EDX analysis

Through the SEM-EDX observations, the structure of individual diatom was observed with more detail chemistry and mineral assemblage. Both *Aulacoseira sp*. and *Navicula oblonga* were covered with thin film on the valve (Fig. 3 to 7). The sectioned diatom valve shows the direct relation between the valve and the thin film (Fig. 3). The *Navicula oblonga* was found covered with mineralic materials (Fig. 4). For the *Aulacoseira sp*. case (Fig. 5), considering the bound film, three different steps are suggested. The first is related to the diatoms valve (a), the second is the thin film which covers the valve (b), and the third is the minerals associated with the film or valve (c). Fig. 6 shows in more detail encrusted valve characteristics. The chemical analyses show that in these three different steps, a substitution process between Si, Fe and Al occured. The Si quantity decreases from the first to the third steps, whereas Fe and Al increase step by step (Fig. 7). Small amounts of Cu and Zn have not shown the similar tedency.

3.5 ICP analysis

The chemistry of the stream water, particularly in the place where the diatoms were found, indicated the presence of some elements as follows: S, 20.42 ppm.; Al, 8.12 ppm.; Fe, 4.49 ppm.; Mn, 0.22 ppm.; Cu, 0.05 ppm.; Cr, 0.05 ppm; Ni, 0.03 ppm and Ti, 0.01 ppm. This place has specifical characteristics of high S, Al and Fe contents due to an isolated branch in the stream system which receives material directly from pond of mining.

4. Discussion

Mineral formation of diatom seems to be controlled by (1) geological structure and mineral composition; (2) water chemistry; (3) pH of the water and (4) the own organisms. Underground water and the non-consolidated sediments are being transported and poured out into the streams where the diatoms occur in the biomat. The non-consolidated sediments contain minerals from the old mine.

In the streams, the pH measurements showed on its average low values, e.g. from 3.6 to 4.5, indicating acidic condition in the water. A seasonality occurence of the biomat was observed. During the spring, summer and autumn, it was easy to be found, showing the maximum distribution and concentration in the streams around the Homanzan mine. During the winter season, however, the distribution of such material was reduced in the places. A similar situation was noted for algae where its seasonal occurence was controlled by ecologic factors: favourable conditions of light intensity, temperature and nutrients (LUNING and DIECK, 1989).

The sediments contain diatoms associated with other organisms as filamentous structures (algae), green-algae and twisted algae. The diatoms distribute under non-saline water condition (ROUND, et al., 1990). In the Homan area, Al, Fe, and S are concentrated. The sediments are red in color, and the mineralogy of the non-consolidated sediments with the biomat is composed of quartz, amorphous materials, hematite, magnetite, akaganeite and feldspar.

The diatoms show an associated relation with thin film on the valve. The *Aulacoseira sp.* case is the best example to explain this occurence. This specimen showed smooth thin and mineral's aggregate films. The chemical composition of the films showed Si, Al, and Fe variations, suggesting the formation process (Fig. 7). The high Si is the major element in the diatoms valve. ROUND et al. (1990) postulated that for diatoms Si has a vital importance on its methabolism. To form valve, the cell wall absorves Si from the environment as the orthosilicic acid ($Si(OH)_4$), and is methabolized as the biogenic silica (amorphous silica). During the cell wall division, new valves will be formed using such material. The diatom valve is classified as a biologically-controlled biomineralization (LOWENSTAN and WEINER, 1989), where the own cell wall controls the mineralization through the influence of organic polymers. In this study the smooth film and mineral's aggregate film show a clear tendency in which the Si quantity decreased, and the Fe and Al increased in the films, depending on the chemistry of acidic stream water. The formation of these thin films can be explained as a biologically-induced biomineralization (LOWENSTAN and WEINER, 1989). One particular characteristic is that the type of minerals formed are a function just as much of environmental conditions. To consider the texture of these two films, LEADBEATER and RIDING (1986) postulate that four physicochemical steps may explain their formation, as follows: (1) solubility of the aqueous solution; (2) super saturation; (3) nucleation; and (4) crystal growth. Also based on LEADBEATER and RIDING (1986), the smooth film represents the step in which the free energy of the phases transformation (from the solution to the mineral) was not enough to form the crystals, remaining the amorphous phase.

The chemistry of the water influence the chemical composition of the valve, smooth film and the minreal's aggregate film which agree with the water's chemistry found in the ICP. The non-visible S in the SEM-EDX analysis may be explained by its high concentration in the water. Depending on the organisms, the own methabolism which decides the kind of element will take part in the chemical composition. Evoking such characteristic, it can explain why S has not taken part in the valve of diatoms. Adaptation, methabolic and defence processes can explain the occurence of the organisms as well as the biominerals formed in the external cell wall.

5. Conclusions

The old copper mine introduce into the environment a polluting effluent composed of non-consolidated sediments and biomat through the underground water. This material is responsible for high concentrations of S, Al and Fe under acidic conditions. The biomat occurence with diatoms in the streams is directly affected by water chemistry with seasonal variations of temperature and illumination. The material bound in the outer part of the cell wall represents biomineralization processes. The interaction between the micro-organism and the inorganic material indicates that the diatom's valve represents a biologically-controlled process of biomineralization.

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