Observation of Microbial Mats from Drainpipe at the Landslide Area in Takayanagi, Niigata, Japan -Part 2 -Occurrence of Various Diatoms-

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Observation of Microbial Mats from Drainpipe at the Landslide Area in Takayanagi, Niigata, Japan - Part 2 - Occurrence of Various Diatoms -

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Abstract - The diversity of microbial mats from drainpipe outlets was found in the landslide area in Takayanagi, Niigata, Japan. High P and Fe were conteined in biomats of drainpipe 1 ~ 3, whereas green biomats in drainpipe 4 mainly contained Si resulted from amounts of diatom occurrence. The diversity of various species of diatom, which capable to indicate water environments, was revealed in different colored biomats. SEM observation of different colored biomats revealed the microbial diversity and their conformation in micron order.

I. Introduction

Landslides are often caused by various factors, such as ground water, artificial environmental change at the place with the geographical feature and the geology-characteristic which causes landslide generating. It had been reported that water qualities affected landslide occurrences [1]. Additionally, measurements of water characteristics have been used way to predict mudslide occurrences [2]. In the landslide area in Takayanagi, Niigata, Japan, the formations of different colored microbial mats have been previously reported [3]. At the locality, the numerous drainpipes were set up to inhibit mudslide occurrence. Each drainpipe carried groundwater resulting the formation of microbial mats in the outlet of the pipes. Where the biomats contained various kinds of diatoms, iron bacteria, tubular, filamentous, coccus, and bacillus types of bacteria associated with inorganic materials [3]. The abundance of particular diatom species in a water sample can indicate nutrient levels and other environmental conditions [4]. Recently, researchers use diatoms to detect the environmental changes in water chemistry or temperature. Additionally, the presence of diatoms with reddish brown materials suggested that compound of Fe in the cells were reported at the study area [3]. Koiwasaki (1996) also reported from a mineralogical study of diatom can take part in the formation of biomminerals associated with Fe, As, and S [5]. Therefore this study is important to understand the differences of environments for iron uptake by diatom. Furthermore, Microbial metabolic reactions have widespread influence on the geochemistry of natural waters through oxidation and reduction of iron and other inorganic ions [6]. For this reason, it might be considered that biomats those consisted of microorganisms, especially diatoms are able to indicate the local environmental conditions.

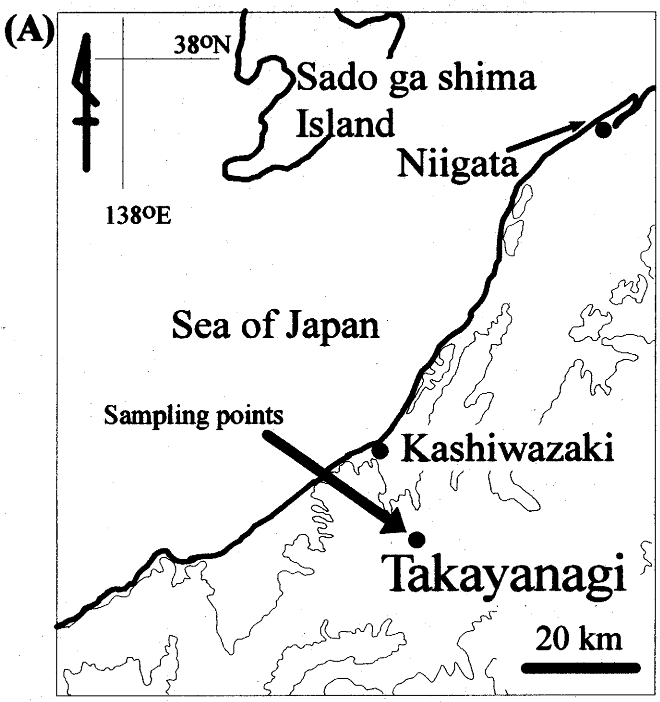
In this study, SEM observations and ED-XRF analyses showed diversity of diatoms in biomats under the different conditions. Furthermore, occurrences of various diatoms from four drainpipes were described.

II. Materials and Methods

Water drainage system (through the pipe) was established in the landslide areas of Takayanagi, Niigata, Japan in March of 1991 (Fig. 1 A). The area is composed of alternation of sandstone and mudstone deposits in Neogene. Near the sampling points, the formations have the axis of anticline, with abundance of paddy fields around there. The numerous drainpipes were set up in the locality to inhibit mudslide occurrence. Each drainpipe carried out groundwater resulting the formation of microbial mats in the outlet of the pipes.

Different colored biomats with ground waters were collected from those drainpipe outlets for analysis on the 13th September 2002 (Fig. 1 B, point 1, 2, 3, and 4). Six drainpipes, 50 m in length, were radially set up at the sampling points for drained groundwater off. Each of the drainpipes has a short interval among them about 50 cm. The biomats are light reddish brown (point 1), reddish brown (point 2), greenish brown (point 3), and green biomats (point 4) in color, respectively.

The ground water qualities are given in TABLE I. The pH was found relatively constant (6.2 ~ 6.5) in groundwater, whereas Eh, DO and WT have increased gradually from point 1 to 4. On the other hand, only EC was found decreased in point 2, 3, and 4 compared with that of point 1. The Eh and DO results showed the relatively oxidative condition in waters between points 1 ~ 4. Specially, point 4 showed the highest oxidative condition of water. Characteristics of water were measured in the field by a portable water quality inspection meter (pH; D-21 made by HORIBA, Eh; D-13 made by HORIBA, EC; ES-12 made by HORIBA, DO and WT: DO21P made by TOA).



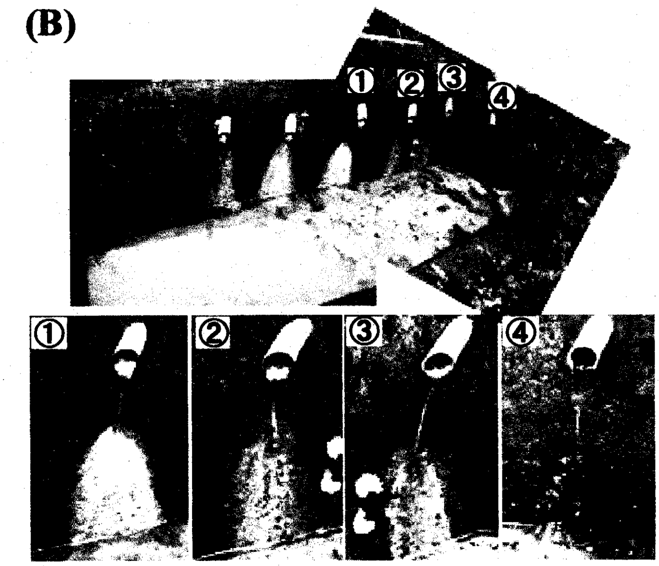


Fig. 1. (A) Locality map of sampling points at the landslide area in Takayanagi, Niigata, Japan. (B) Field views of biomats in drainpipe outlet at the landslide area in Takayanagi showing light reddish brown biomats (①), reddish brown biomats (②), greenish brown biomats (③), and green biomats (④).

The chemical compositions of biomats analyzed by an energy dispersive X-ray fluorescence spectrometer (ED-XRF; JEOL JSX 3201 using Rh Kα). Each of the biomat samples was mounted on the Mylar film. After air-drying,they were measured at an accelerating voltage of 30 kV under a vacuum condition.

To identify the species and number of diatoms, treatments were carried out for biomats from point 1 to 4 in a manner

TABLE I

Characteristics of water collected from drainpipe at the landslide area in Takayanagi, Niigata, Japan.

Sampling point	pH	Eh(mV)	EC(mS/m)	DO(mg/l)	WT(℃)
1	6.5	141	29.8	2.1	13.0
2	6.4	190	22.3	3.4	14.9
3	6.2	303	19.4	3.8	15.3
4	6.3	456	25.8	6.7	17.2

13th September 2002, mesurement

similar to Yanagisawa (1999) [7]. After air-drying, 1 g biomats were suspended in distilled water of 100 ml. The diatoms in 0.5 ml suspensions pipetted on to glasses were observed. Abundances of diatoms were shown as VA (very abundant), C (common), and R (rare).

The scanning electron miroscope equipped with an energy dispersive X-ray spectroscopy (SEM-EDX; JEOL JSM-5200 LV and PHILLIPS EDAX PV 9800 EX) was used in order to observe the micro morphological surface of microorganisms and its chemical composition. After freeze-drying, the surface were coated with carbon and examined at an accelerating voltage of 15 kV.

III. Results

The ED-XRF analysis of bulk biomats collected from sampling point 1 to 4 in Fig. 1 B revealed that they consisted mainly of P, Fe, Si, and trace of S (Fig. 2). Minor contents of Na, Mg, Al, K, Ca, Ti, Mn, As, and Ba were also detected. Fe was rich (45 ~ 70 wt%) in point 1 (light reddish brown), point 2 (reddish brown), and point 3 (greenish brown). Additionally, biomats in point 1 (light reddish brown) was rich in P-Fe (both of them about 45 wt% contents). For comparison, green biomats in point 4 was rich in Si (about 80

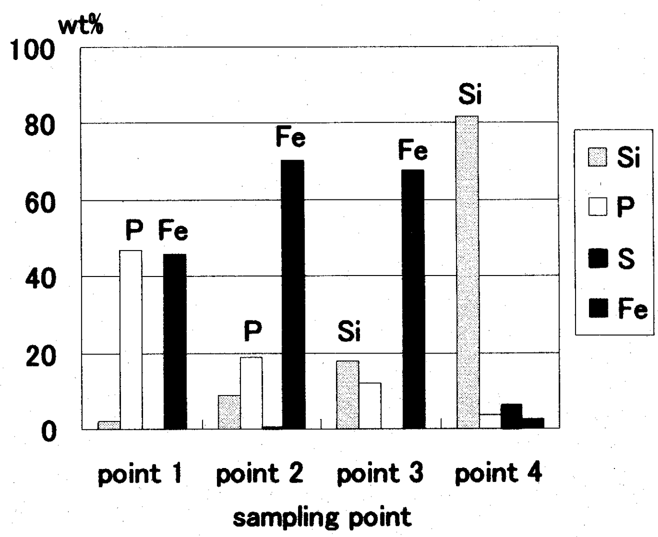


Fig. 2. Main chemical components of biomats in drainpipe outlet at the landslide area in Takayanagi.

wt%). The reverse correlation was found between P and Si contents. The P content has decreased whereas Si content has increased from sampling point 1 to 4 gradually. On the other hand, S content of green biomats in point 4 was relatively high (about 5 wt%) compared with other biomats in point 1 ~ 3.

Species and amounts of diatoms in each of the biomats were shown in TABLE II. Diversity of various diatom species was revealed in biomats of point 1 ~ 4. *Nitzschia* sp. A (cf. *palea*) is the most abundant in all sampling points, such as 100 in point 1, 60 ~ 99 in point 2 and 3, and 44 in point 4. *Gomphonema* sp. was found in only point 2, whereas *Napicula* sp. (aff. *mutica,* minute) and *Nitzshia* sp. B (rectangular, large) were found in only point 4. Additionally, occurrence of *Achnanthes* sp., *Frustulia* cf. *vulgaris, Nitzhia* spp. (small), and *Pinnularia* spp. (large) were also revealed in the biomats in point 2 ~ 4. Numbers of diatom valves per slides were increased gradually from point 1 to 4. Especially, numerous diatoms were found in the point 4.

SEM observations of different colored biomats revealed diversity of microorganisms and their conformation in micron order. The details are as follows according to the sampling points. In the point 1, light reddish brown biomats consisted mainly of numerous filamentous types of bacteria adhered with small particles (Fig. 3 ①-a). Magnified view of Fig. 3 ①-a shows filamentous types of bacteria aggregate and forms the colonies (Fig. 3 ①-b). The arrow in Fig. 3 ①-b indicates the presence of P, Fe, and traces of Cl and Ca on the cell surface of filamentous types of bacteria (inset Fig. 3 ①-b). Additionally, diatoms observed by SEM were only *Nitzschia* sp. (cf. *palea*) in point 1 (Fig. 3 ①-c). Micrograph of the reddish brown biomats in point 2 shows *Leptothrix ochracea* formed network structure associated with filamentous types of bacteria and flossy materials (Fig. 4 ②-a). Diatoms were covered with filamentous types of bacteria (Fig. 4 ②-b). The surface of *Leptothrix ochracea* was mainly composed of Fe, P, Si, and traces of Cl and Ca (inset Fig. 4 ②-a), whereas Si and Fe were detected from surface of the diatom (inset Fig. 4 ②-b).

TABLE II

Occurrence chart of diatom species in biomats collected from drainpipe at the landslide area in Takayanagi.
Abundance, VA; very abundant, C; common, R; rare. + : present

Nonmarin Diatoms (benthic)		Sampling point 1	2	3	4
app. Number of diatom valves per slide (×100)		27	36	108	360
Abundance		R	R	C	VA
Achnanthes	sp.		5	+	7
Frustulia	cf. vulgaris Thwaites		3	1	
Gomphonema	sp.		32		
Navicula	sp. (aff. mutica, minute)				29
Navicula	spp. (small)			+	
Nitzschia	sp. A (cf. palea (Kuetz.) W. S.)	100	60	99	44
Nitzschia	sp. B (rectangular, large)			+	20
Nitzschia	spp. (small)				+
Pinnularia	spp. (large)				+
Total number of diatoms counted		100	100	100	100

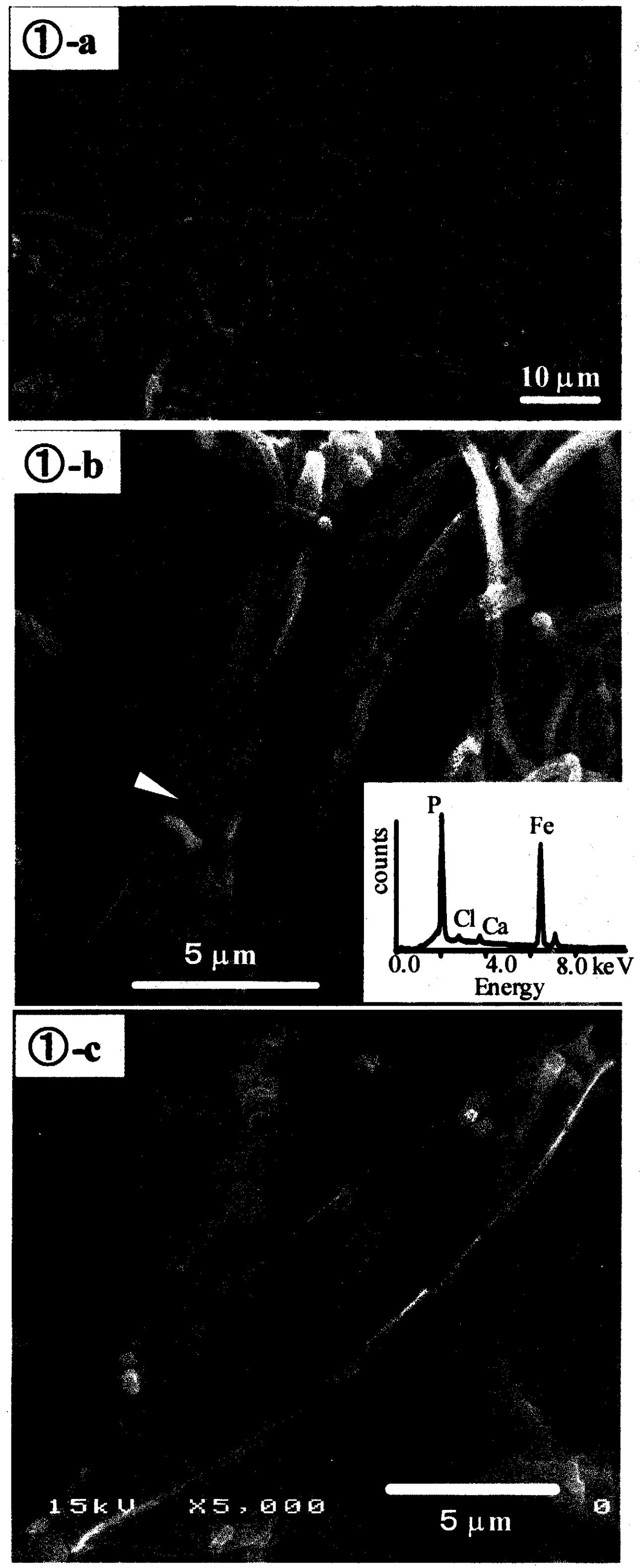


Fig. 3. Scanning electron micrographs and the EDX analysis of light reddish brown biomats in point 1. (①-a) Filamentous bacteria formed network structure. (①-b) Magnified view of ①-a showing colonies of filamentous types of bacteria. EDX spectrum of the filamentous bacteria shows strong peaks of P and Fe: inset (analytical point: arrow in ①-b). (①-c) The presence of *Nitzschia* sp. were revealed.

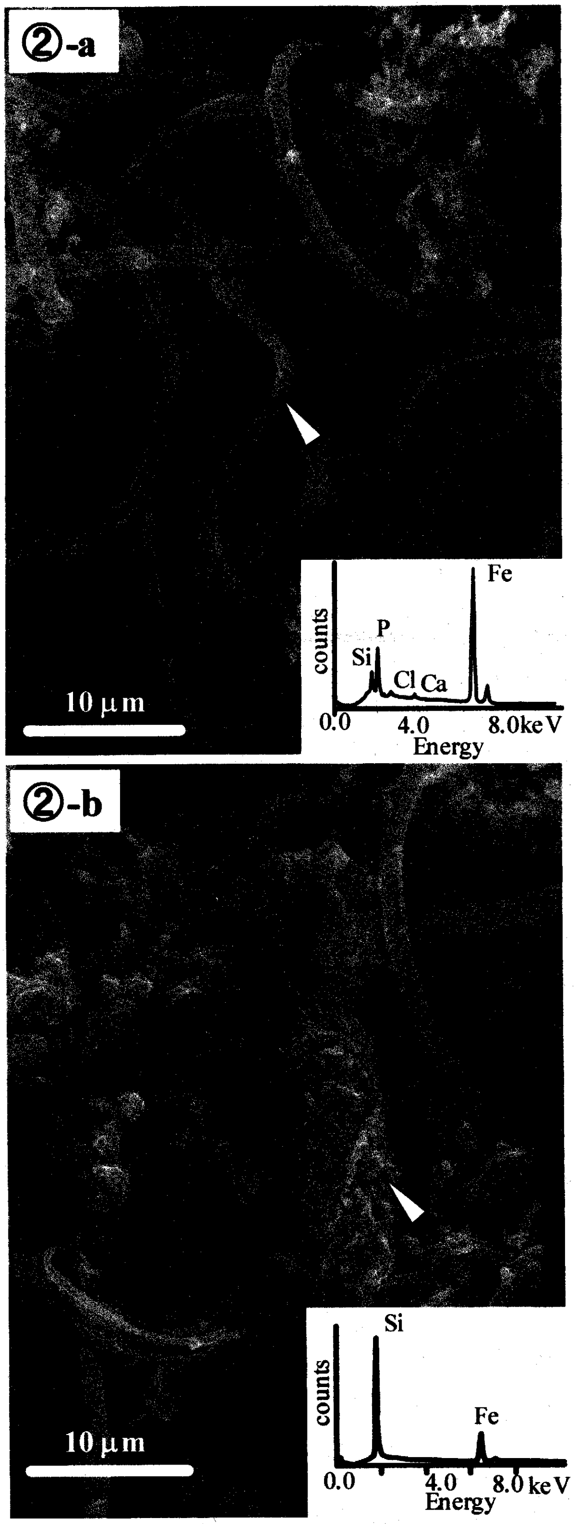


Fig. 4. Scanning electron micrographs and the EDX analyses of reddish brown biomats in point 2. (②-a) *Leptothrix ochracea* with flossy materials formed network structure. EDX spectrum of the surface of *Leptothrix ochracea* shows strong peaks of Fe, P and trace of Si, Cl, and Ca: inset (analytical point: arrow in ②-a). (② -b) Diatoms were covered with filamentous types of bacteria. EDX spectrum of the surface of diatom shows strong peaks of Si and Fe: inset (analytical point: arrow in ②-b).

In point 3, tubular types of bacteria associated with *Leptothrix ochracea.* Especially, numerous tubular types of bacteria covered on the diatom cells (Fig. 5 ③). Fe, Si, P, and trace of Cl were found on surface of *Leptothrix ochracea* (Fig. 5 ③ A), whereas high concentration of Si and Fe were detected from the surface of diatom covered with tubular types of bacteria (Fig. 5 ③ B). On the other hand, SEM observation of the green biomats from point 4 revealed the presence of various kinds of diatoms associated with cyanobacteria (Fig. 6 ④-a). EDX spectrum of diatom surface indicated high concentration of Si (inset Fig. 6 ④-a). Diatoms were covered with mucus layer-like thin film (Fig. 6 ④-b). Diatom contained spherical material inside of the cell (Fig. 6 ④-d).

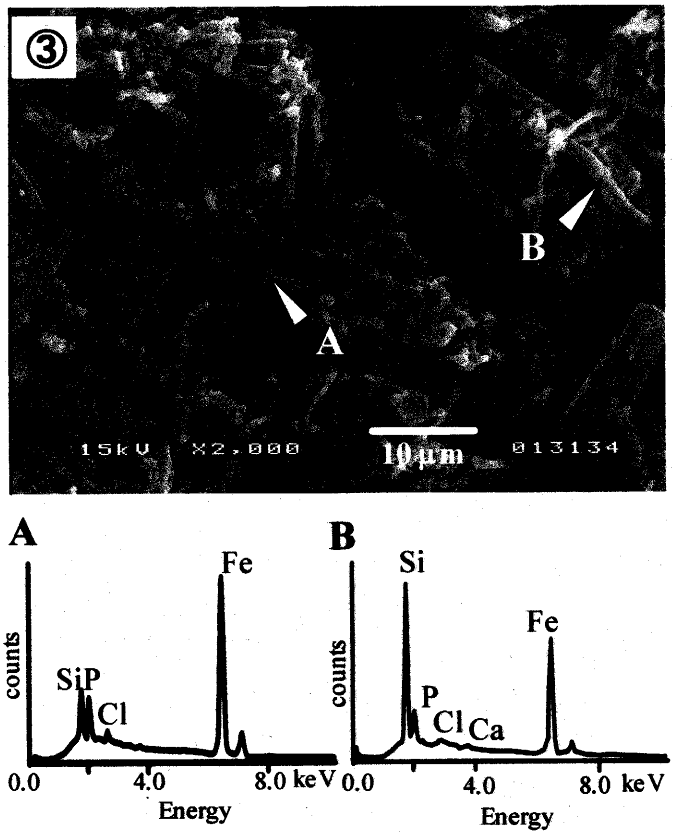


Fig. 5. Scanning electron micrographs and the EDX analyses of greenish brown biomats in point 3. (③) Numerous tubular types of bacteria associated with *Leptothrix ochracea* covered on the diatom cells .(A) EDX spectrum of the surface of *Leptothrix ochracea* shows a strong peak of Si, P and Fe. Analytical point: arrow in ③ A. (B) EDX spectrum of the surface of diatom with tubular types of bacteria shows high concentration of Si and Fe. Analytical point: arrow in ③ B.

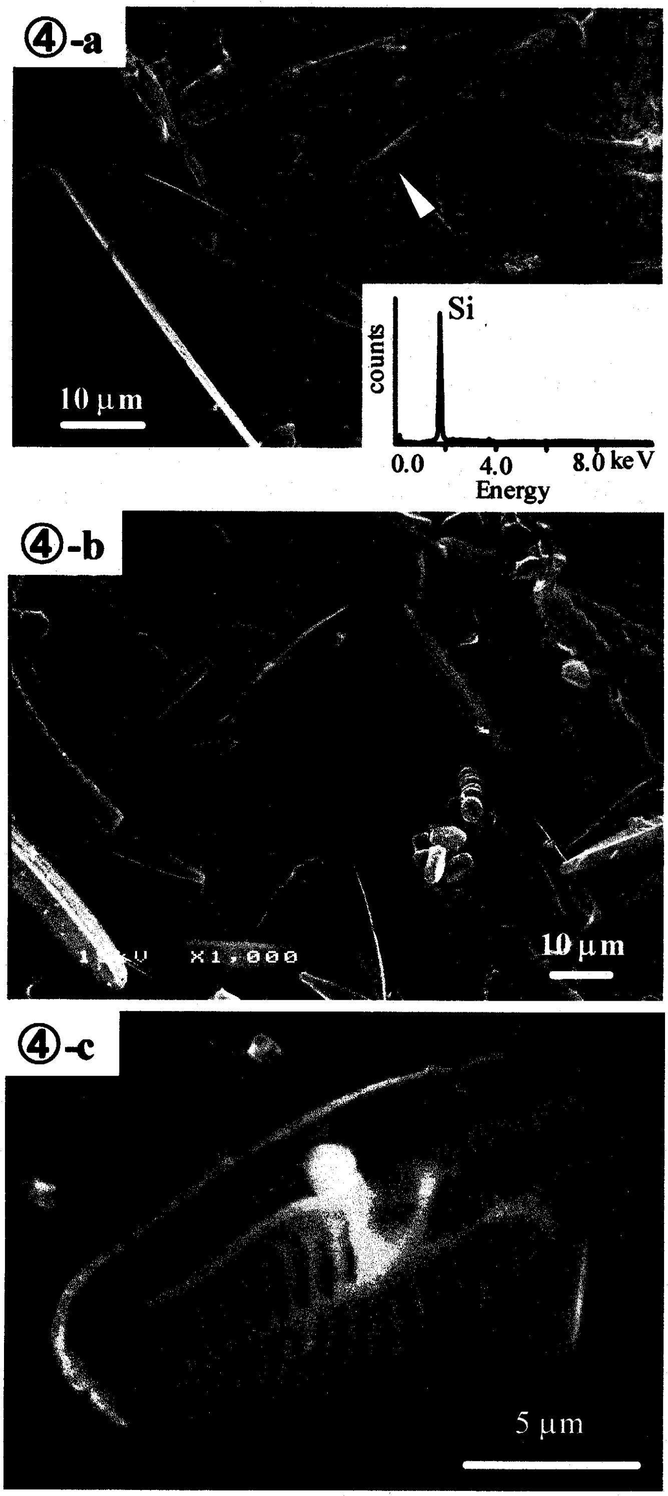


Fig. 6. Scanning electron micrographs and the EDX analysis of green biomats in point 4. (④-a) General view of green biomats shows presence of various kinds of diatom and cyanobacteria. EDX spectrum of diatom surface shows a strong peak of Si: inset (analytical point: arrow in ④-a). (④-b) Diatoms were covered with thin film. (④-c) Diatom contained spherical material inside of the cell.

IV. Discussion

In this study, accumulation of P and Fe in biomats of points 1 ~ 3 and Si accumulation in green biomats of point 4 were found (Fig. 2). High Si contents conformed to the number of diatoms. Difference of biomats components suggested variety of water quality originated from under ground conditions as bleeding channels. Therefore various groundwater qualities might be affected by landslide terrain. Additionally, high content of P (points 1 ~ 3) might be reflected in eutrophied environments of the water resource. The needle-like, extracellular precipitates of magnetotactic bacteria were reported as by-products after the metabolic release of ferrous iron into a phosphate-rich medium [8]. Consequently, this study is also assumed quite interesting work from biomineralogical stance.

Nitzschia palea has been reported as an indicator of the polluted area [9]. At point 1, only *Nitzschia* sp. (cf. *palea*) was detected. On the other hand, *Napicula* sp. (aff. *mutica*, minute), which often appeared in relatively pure water systems, was observed only in green biomats of point 4. The results suggested that point 4 was relatively pure environmental conditions compared with point 1.

Observation of biomats revealed the diversity of environmental ecosystem was found in the landslide area of Takayanagi, Niigata, Japan. In point 2 and 3, numerous filamentous and tubular types of bacteria associated with *Leptothrix ochracea* covered on diatoms (Fig. 4 ②-b and Fig. 5 ③). *Leptothrix ochracea* deposit copious amounts of iron hydroxide as encrusted sheaths in the various environments where they grow [10]. Therefore these bacteria were presumed one of iron bacteria as a result of elemental analysis (ED-XRF and EDX). The reason of these bacterial covering on to the diatoms might be the oxidative condition of diatom cell surfaces, which is capable to accelerate iron oxidation. Diatoms accreted with these aerobic bacteria, filamentous and tubular types of bacteria, which might prefer oxygenic condition compared with *Leptothrix ochracea*. In point 4, diatom was observed with the spherical material in their cells (Fig. 6 ④-c). The presences of reddish brown materials inside of diatom cells have observed in previous report [3]. The spherical material might be same as the reddish brown materials suggested Fe compounds taken up by diatom.

In this study, the diversity of microbial mats from drainpipe outlets was found in the landslide area in Takayanagi, Niigata, Japan. Microorganisms, such as iron bacteria, diatom, and cyanobacteria, in microbial mat certainly indicated difference of groundwater conditions, which might be affected by landslide terrain.

V. Conclusion

The diversity of environmental ecosystems in drainpipe outlet was found in the landslide area in Takayanagi, Niigata Japan. The accumulations of P and Fe in biomats of points 1 ~ 3 were revealed. In point 4, green biomats mainly composed of Si resulted from amounts of diatom occurrence. The diversity of various species of diatom was revealed from point 1 to 4. Varieties of diatom suggested difference of environmental condition in a short interval. Numbers of diatom valves per slides were increased gradually from point 1 to 4. SEM observation of different colored biomats revealed the microbial diversity and their conformation in micron order.

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