Analysis of Antarctic Soil Algae by the Direct Observation Using the Contact Slide Method

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スライドグラス埋設法により南極土壌から検出された藻類

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要旨: 南極ラングホブデ雪鳥沢において，スライドグラス埋設法を用い，土壌含水量と関連させ，土壌藻類の種類組成とその分布を調査した．沢の5カ所において，沢の流れに直交する方向に数m間隔で3～6の地点，スライドグラスを垂直に1988年1月より翌年1月まで埋設した．

種類としては，11種類の藍藻類，8種類の緑藻類，4種類の珪藻類が検出された．それらのうち，*Actinotaenium cucurbita* と *Pinnularia borealis* がしばしば優占種として出現した．走査型電子顕微鏡による観察では，珪藻類は薄い粘液を分泌し，糸状性藍藻類の *Lyngbya martensiana* は厚い粘液を分泌してスライドグラスに固着していた．

藻類の種類数，細胞数及び土壌中のクロロフィル量は土壌の含水量と強い関連が見られた．これらはいずれも土壌含水量が高い沢近くの地点では多く，沢から数m離れると土壌含水量は激減し，そこでは著しく少なくなった．

Abstract: The species composition and distribution of soil algae *in situ* were investigated by the direct observation using the contact slide method with reference to the available water in soils of the Yukidori Valley, Langhovde, Antarctica. Glass slides were vertically buried at five sites with a few meters intervals along a stream for a year from January 1988.

Eleven taxa of Cyanophyceae, eight taxa of Chlorophyceae and four taxa of Bacillariophyceae were recognized on the collected glass slides. *Actinotaenium cucurbita* (RALFS) TEILING and *Pinnularia borealis* EHRENB. were often dominant. The results of the observation by the scanning electron microscope showed that diatoms secreted thin mucilage, but a filamentous blue-green alga, *Lyngbya martensiana* MENEGH. secreted thick mucilage and adhered to the surface of glass slides.

Both the species number and the algal cell number of the soil algae and the chlorophyll content in soils were well related to the water content of soils. Values of these three items were high at the nearest transect points to the stream where the water content of soils was high, and suddenly decreased at a few meters distance from the stream where the water content of soils was low.

1. Introduction

Algal colonies developed on the surface and within the shallow part of soils in the Antractic continent. The dilution method has been commonly used for the counting of microbes of soil or mosses in the Antractic (CAMERON and DEVANEY, 1970; BROADY,

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1979a, b). Dilution method, however, is not suitable for the ecological study of species which are difficult to grow under the culture conditions. The chlorophyll content of soil has been also applied to estimate the algal abundance (HOSHIAI and MATSUDA, 1979; FRIEDMANN *et al.* 1980; BROADY, 1986; AKIYAMA *et al.*, 1986). With the pigment extraction method, it is difficult to know the species composition of algae and to distinguish the pigments of algae from the pigments of other plants. On the other hand, the contact slide method has been known as a convenient method for the direct counting of soil microbes and for the identification of the soil algae under the condition *in situ* (CHOLODNY, 1930; PIPE and CULLIMORE, 1980). PIPE and CULLIMORE (1980) identified the soil algae to the genus level such as *Chlorella*, *Hantzschia*, *Oscillatoria*, etc. In order to obtain the details of distribution of the soil algae *in situ* with reference to available water in soils, we studied the species composition of the soil algae and measured the algal cell number by the direct observation using the contact slide method, namely, the soil algae on the glass slides buried in Antarctic soils were investigated.

The present study was conducted as part of the four-year research project "The studies on the mechanism of Antarctic terrestrial ecosystems" from 1986 to 1990 (JARE-27~31).

2. Materials and Methods

Five sites for this study were chosen along the stream in the Yukidori Valley, Langhovde, Antarctica about 20 km south of Syowa Station (Fig. 1). The valley is about 2.5 km long, and runs from east to west. It contacts the continental ice sheet at the east end, and the shore of Lützow-Holm Bay at the west end. A melt

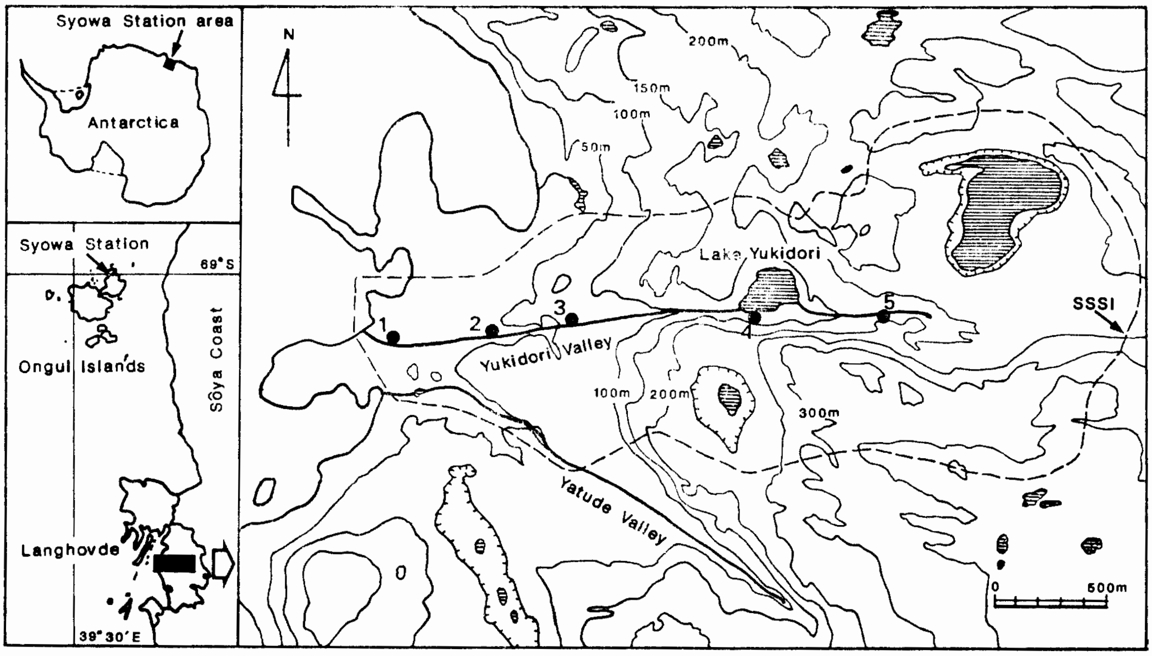

Fig. 1. Map showing the sites studied by the contact slide method in the Yukidori Valley, Langhovde, Antarctica.

stream flows along the valley during the austral summer. At each site, we chose several points as a suitable place for setting a glass slide at a few meters intervals along a line transect which was set across the stream in order to survey the relation of both species composition and cell number of the soil algae to the available water in soils (Fig. 2: 1–3). Vegetation and substrata of the sites are shown in Table 1.

The translucent frosted glass slides (*ca.* 76×26 mm) were vertically buried mainly in sandy soils at the chosen points for a year from middle January 1988 (Fig. 2: 4, arrow). The top portion of glass slides corresponds to the surface of soil. The light may penetrate into the deeper part of the soil through the top surface of glass slide than the real depth in the field condition. Among sites 1, 2 and 3 which were used for the quantitative study of soil algae, glass slides of site 2-E, site 3-I and site 3-H were lifted 3 mm, 3 mm and 10 mm, respectively by freeze and thaw cycle of soils. After collection of the glass slides, they were kept in a freezer (−20°C) until ready for examination.

Samples were observed for the identification using the light transmission microscope (Nikon, OPTIPHOT) with magnification up to ×1000. Two samples were examined by the scanning electron microscope (JEOL, JSM-5200) to observe detail features of algal cells on the glass slide. A part of samples (5 mm×5 mm) was dried at room temperature in the desiccator. After gold coating, they were observed by SEM with magnification up to ×2000.

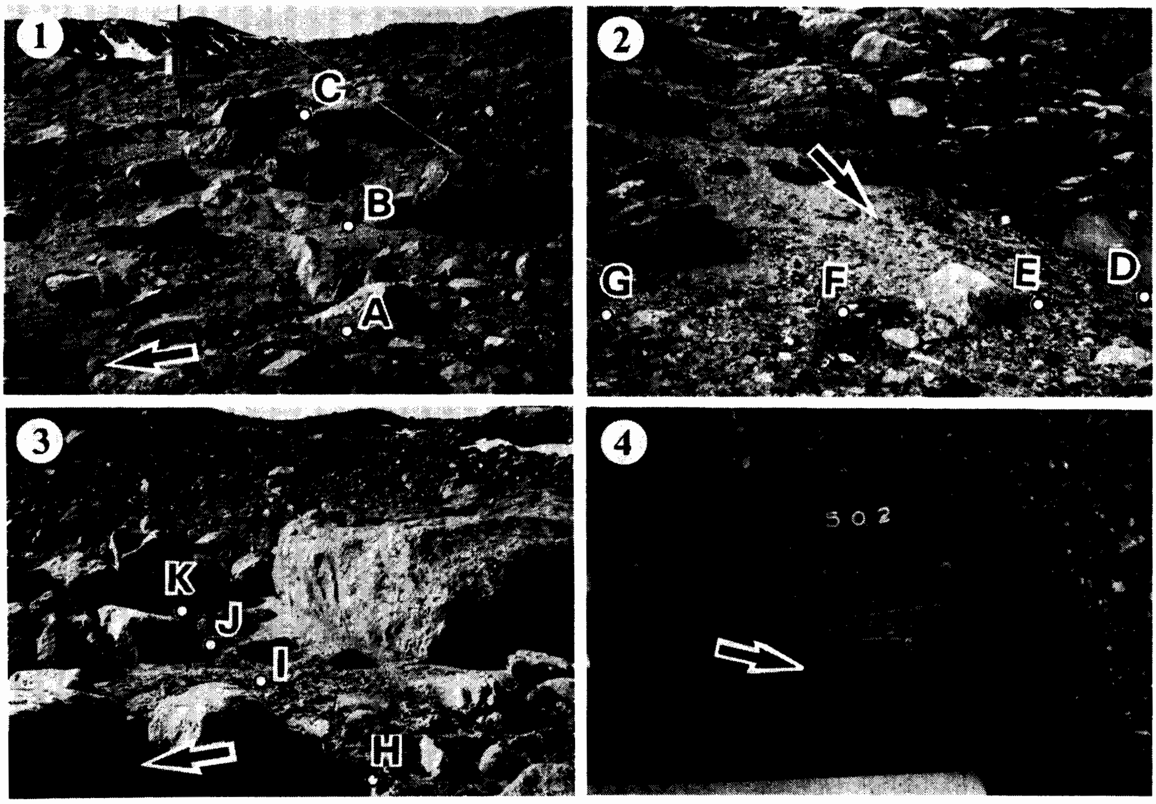

Fig. 2. Line transect of the five sites and setting of frosted glass slide. 1: Site 1. 2: Site 2. 3: Site 3. (arrow showing the direction of lower course in Fig 2: 1–3). 4: A frosted slide glass (arrow) vertically buried in the soil at each transect point.

Table 1. Vegetation and substrata of the studied sites.

Site No.		Vegetation	Substrata
Site 1			
A	(0.5 m)*	Moss	Soil
B	(3.5 m)	Moss	Soil
C	(6.5 m)	—	Soil
Site 2			
D	(0.3 m)	Moss	Soil
E	(1.6 m)	Moss	Soil
F	(2.9 m)	Moss	Soil
G	(4.2 m)	—	Soil
Site 3			
H	(0.0 m)	Moss	Soil
I	(1.5 m)	Moss	Soil
J	(3.0 m)	Moss	Soil
K	(4.5 m)	Moss	Soil
Site 4			
L	(0.1 m)	Moss	Soil
M	(1.6 m)	Moss	Soil
N	(3.1 m)	Moss	Soil
O	(4.6 m)	Moss	Soil
P	(6.1 m)	—	Soil
Q	(7.6 m)	—	Soil
Site 5			
R	(0.0 m)	Algae	Soil
S	(1.5 m)	Algae	Soil
T	(3.0 m)	Moss	Moss
U	(4.0 m)	Moss	Moss
V	(5.0 m)	—	Soil

* Distance from the stream in middle January 1988. —: No vegetation.

Algal numbers on glass slides were counted using the reflected light microscope (OLYMPUS) at the depth of 0.5, 1, 2, 3 and 5 cm, respectively. A filamentous blue-green alga, *Lyngbya martensiana* MENEGH. occurred abundantly at site 2-D, but, cell count was not achieved because of the difficulty of accurate count of cell number. A colony of *Nostoc* was counted as one cell.

The water condition of soils was recorded with three ranks such as dry, moist and wet on January 1988, while soils and mosses at the surface and at the 5 cm depth were sampled at each site on January 1989. The water content of soils and mosses was expressed by the following formula:

Water content (%)=(Wet weight-Dry weight)/Dry weight $\times$100.

The chlorophyll *a* content of the soil was measured according to the standard method by UNESCO (1966).

Analysis of the species composition was achieved with samples from all five sites, but measurements of the water content of soils, cell number of soil algae and the chlorophyll content were done with samples from sites 1, 2 and 3.

3. Results

3.1. Soil algae detected on the glass slide

Eleven taxa of Cyanophyceae, eight taxa of Chlorophyceae and four taxa of Bacillariophyceae were recognized on the collected glass slides (Table 2). Among them, *Actinotaenium cucurbita* (RALFS) TEILING and *Pinnularia borealis* EHRENB. were often dominantly occurred. *Stigonema minutum* (Ag.) HASSALL, *Gloeocapsa magma* (BRÉB.) HOLLERBACH, *Gloeocapsa ralfsiana* (HARV.) KÜTZ. and a filamentous green alga were detected from the glass slide buried in a moss colony (Site 5-T). Other algae were detected from the glass slides buried in soils. Most of blue-green algae and diatoms were identified to the specific level because modification of cell shape by the preservation in a freezer and the desiccation was so small (Fig. 3: 1, 2). Although eight taxa of green algae were found, they were not identified to the genus level except *Actinotaenium* and *Stichococcus*, because cell contents of them were not clear (Fig.

Table 2. Soil algae detected from the suface of the glass slides buried in the five sites along the Yukidori Valley.

Species	Site				
	1	2	3	4	5
Cyanophyceae					
Synechococcus aeruginosus NÄG.	●	●	—	●	●
Synechococcus maior SCHR.	●	●	●	●	●
**Gloeocapsa magma* (BRÉB) HOLLERBACH	—	—	—	—	●
Gloeocapsa ralfsiana (HARV.) KÜTZ.	●	—	—	—	●
**Gloeocapsa compacta* KÜTZ.	—	—	—	—	●
Lyngbya martensiana MENEGH.	—	●	—	●	●
Lyngbya sp. 1 (width = 1 μm)	—	●	—	—	—
Lyngbya sp. 2 (width = 3 μm)	—	—	—	—	●
Nostoc commune VAUCHER	●	—	—	—	—
Nostoc sp.	●	●	—	●	—
**Stigonema minutum* (AG.) HASSALL	—	—	—	—	●
Bacillariophyceae					
Hantzschia amphioxys (EHRENB.) GRUN.	●	●	●	●	—
Navicula muticopsis VAN HEURCK	●	●	●	●	—
Navicula sp.	●	—	—	—	—
Pinnularia borealis EHRENB.	●	●	●	●	●
Chlorophyceae					
Actinotaenium cucurbita (RALFS) TEILING	●	●	●	—	—
Stichococcus sp.	—	—	—	●	—
Coccoid alga no. 1 (diam. = 10 μm; tetrads)	—	●	—	—	—
Coccoid alga no. 2 (diam. = 10–16 μm; unicell)	—	—	—	●	—
Coccoid alga no. 3 (diam. = 6–8 μm; colonies with mucilaginous substance)	●	—	—	—	—
Coccoid alga no. 4 (irregularly shaped colonies composed of densely aggregated cells)	—	—	—	●	—
Coccoid alga no. 5 (colonies of 1, 2, 4 to 16 cells with thick mucilaginous substance)	—	●	—	—	—
*Filamentous alga	—	—	—	—	●

* Occurred in moss colonies.

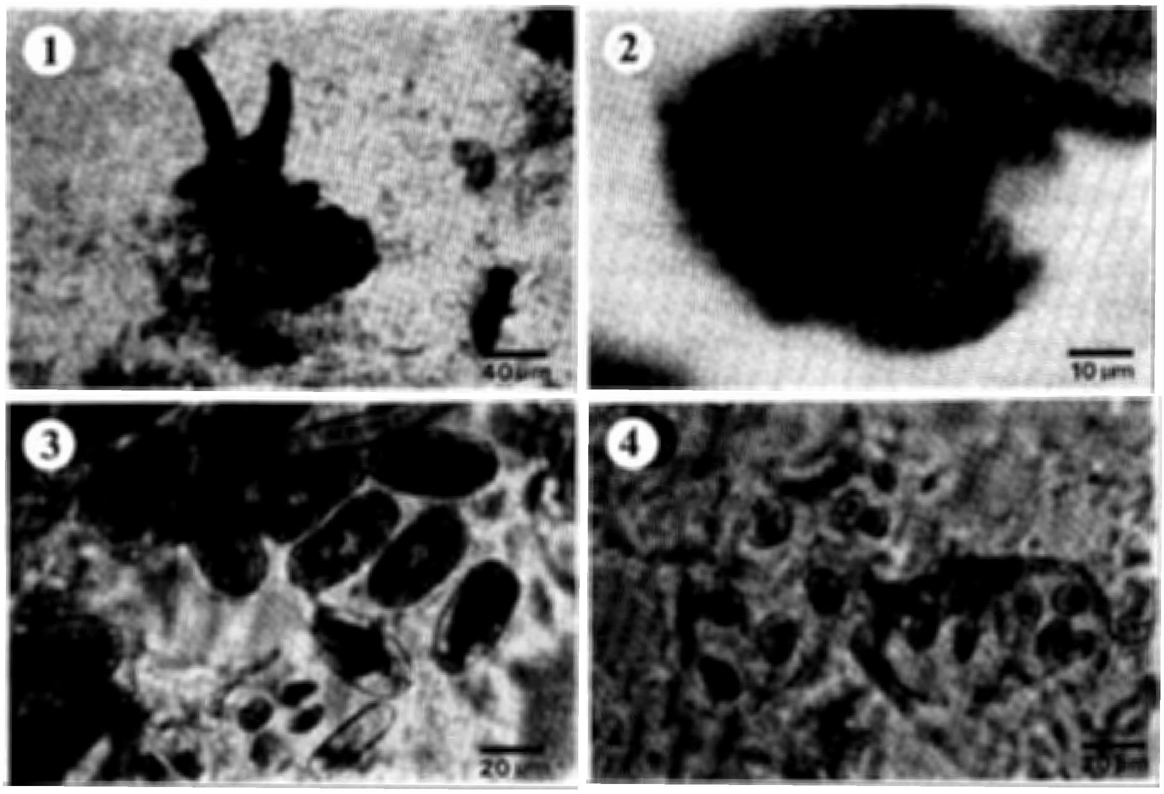

Fig. 3. Soil algae on the glass slides observed by the light microscope. 1: Filaments of Stigonema minutum. *2: A colony of* Gloeocapsa ralfsiana. *3: Cells of* Actinotaenium cucurbita. *4: Colonial green algae with thick mucilaginous substance.*

3: 3, 4). Other coccoid or filamentous green algae need the culture examination for their identification.

Detail features of the algal cells on the glass slides were observed by the scanning electron microscope (Fig. 4). A filamentous blue-green alga, *Lyngbya martensiana* MENEGH. adhered to the glass slide with thick mucilaginous substance. Unicellular algae were sometimes wrapped in the mucilage of *L. martensiana* (Fig. 4: 2, arrow). *Synechococcus maior* NÄG, and three diatoms were attached to the glass slide with thin, and unapparent mucilages (Fig. 4: 3–6). It is observed by the light transmission microscope that colonies of green algae adhered on the surface of the glass slide with thick mucilage (Fig. 3: 4) as well as filamentous blue-green algae.

3.2. The distribution of the soil algae

The water condition of soils and mosses, the species number on a glass slide, the mean algal cell number (cells/mm^2) on a glass slide and the chlorophyll *a* content in the soil at sites 1, 2 and 3 along the stream are shown in Table 3.

During the austral summer 1988, meltwater flowed in the stream from the upper course to lower course. At site 1-A, site 2-D and site 3-H which are the nearest transect points to the stream, the water condition on January 1988 was moist or wet. The water condition showed a tendency to change from wet, moist to dry with the distance from the stream. At site 1-C, site 2-G and site 3-K, the farthest transect

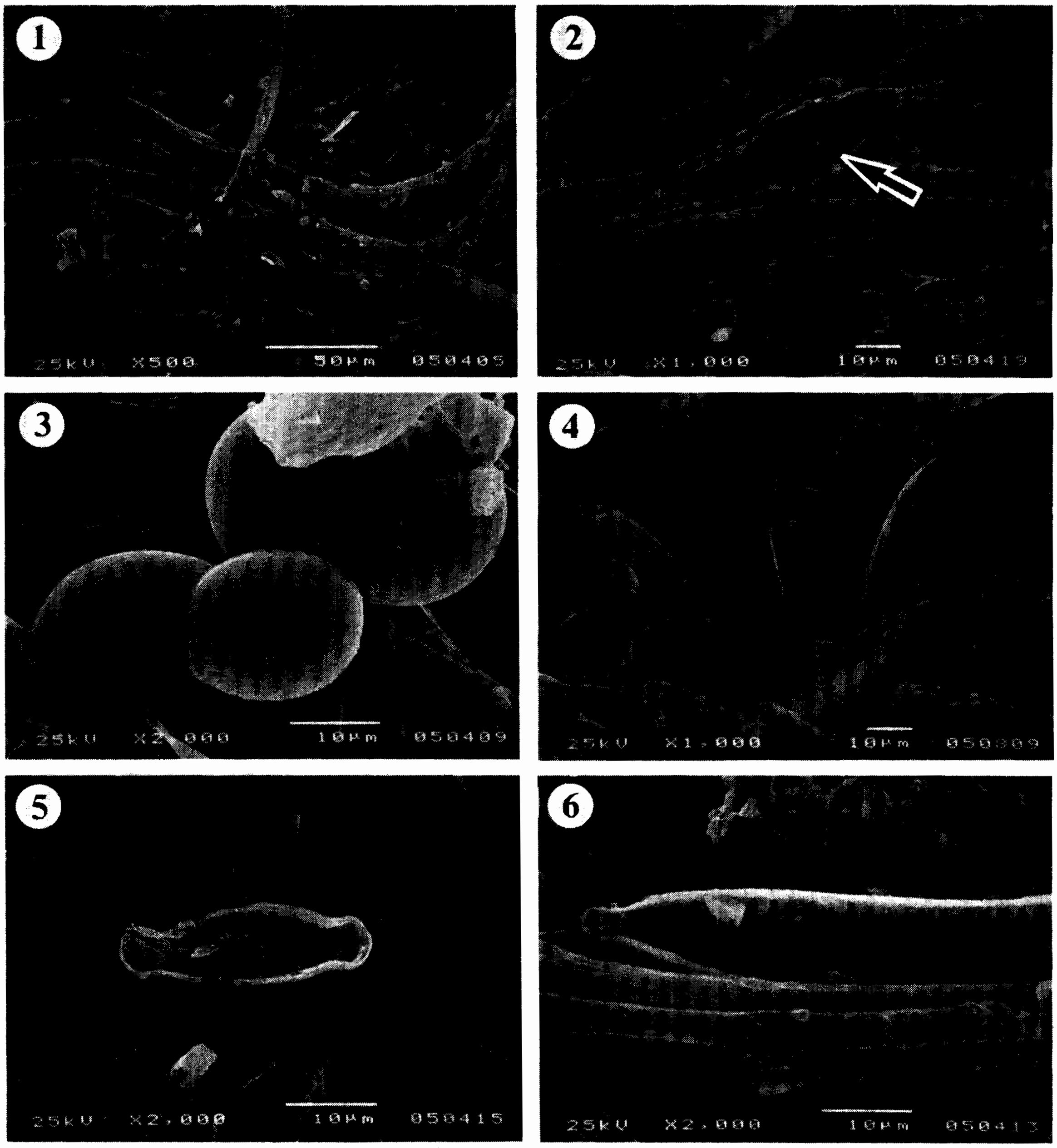

Fig. 4. Soil algae on the glass slides observed by the scanning electron microscope. 1: Lyngbya martensiana *adhered to the surface of the glass slide by mucilaginous substance. 2:* Pinnularia borealis *(arrow) wrapped in mucilaginous substance of* L. martensiana. *3: Cells of* Synechococcus maior. *4: A colony of* Pinnularia borealis. *5:* Navicula muticopsis. *6:* Hantzschia amphioxys.

points from the stream, the water condition was dry except at 5 cm depth of site 2-G. During the austral summer 1989, meltwater hardly ran in the stream from the west margin of Lake Yukidori to the mouth of the stream, and the water was supplied mainly from snow drifts. The water content on January 1989 ranged from 0.1 to 30.9%. It decreased suddenly at a few meters distance from the stream at site 1 and site 3. At site 2, water condition was low and almost the same among all the transect

Table 3. Horizontal change of the water condition of soils, the species number on a glass slide, the mean algal cell number on a glass slide and chlorophyll a content in soils at sites 1, 2 and 3.

Site No.	Water condition				Species number	Mean algal cell number (cell/mm²)	Chlorophyll content (μg/g)
	January 1988		January 1989				
	Surface	−5 cm	Surface (%)	−5 cm (%)			
Site 1							
A (0.5 m)	Moist	Moist	30.9	21.6	10	41	18.6
B (3.5 m)	Dry	Moist	0.1	1.7	3	2	3.5
C (6.5 m)	Dry	Dry	<0.1	1.1	1	<1	1.9
Site 2							
D (0.3 m)	Wet	Wet	0.1	0.2*	9	212	23.5
E (1.6 m)	Dry	Moist	0.1	0.2	4	<1	2.4
F (2.9 m)	Dry	Moist	0.1	0.3	1	<1	—
G (4.2 m)	Dry	Moist	0.1	0.5	1	<1	—
Site 3							
H (0.0 m)	Wet	Wet	1.1**	2.5	5	316	9.5
I (1.5 m)	Moist	Moist	1.6**	1.2	1	<1	9.4
J (3.0 m)	Dry	Dry	0.1	0.9	0	0	—
K (4.5 m)	Dry	Dry	0.1	0.3	0	0	—

* Depth is 2.5 cm. **Water content of moss colony. —No data.

points, because little water was supplied from snow drifts.

The species number of the soil algae was well correlated with the water contents of soils and mosses as shown in Table 3. For example, at site 1-A (0.5 m distant from the stream) where the water content was high, ten species were found. On the other hand, at the transect point site 1-C (6.5 m distant from the stream) where the water content of soil was quite low, only one species was found.

At sites 1, 2 and 3, the algal cell number and the chlorophyll content of the soil were measured. The mean algal cell number ranged from 0 to 316 cells/mm². The chlorophyll content ranged from 1.9 to 23.5 μg/g. The results suggested that the algal cell number and the chlorophyll content were well correlated with the water content of soils as well as the number of species. Highest values of them were recorded at the nearest transect points to the stream where the water content of soils was high. The cell number obtained by the contact slide method was closely related to the values of chlorophyll content of the soil which were derived from mainly episammic algae at the same site ($r=0.75$).

Vertical change of algal cell number at sites 1, 2, and 3 is shown in Table 4. The occurrence of soil algae on the surface of each glass slide was recognized between the top portion of the glass slides which almost corresponded to the soil surface and the lower portion of the glass slide corresponding to the soil of *ca.* 7 cm depth. The majority of algae occurred on the glass slide less than 5 cm deep. The structure of algal community, particularly of their vertical micro-distribution, varied with sites, *viz.* the depth showing a maximal algal growth at each site was as follows: −0.5 cm (53 cells/mm²) at site 1, −1 cm (318/mm²) at site 2 and −2 cm (555 cells/mm²) at site 3 (Fig. 5). Among species shown in Table 4, the cell number of *Pinnularia borealis* was highest and its cells occurred at 5 cm depth of site 1-A and site 3-H. The remark-

Table 4. Vertical change of algal cell number (cells/mm²) on the glass slides at the line transects of sites 1, 2 and 3.

Site No.		Algae	Depth (cm)				
			0.5	1	2	3	5
Site 1	A (0.5 m)	*Actinotaenium*	34	13	5	2	0
		Pinnularia	19	21	32	29	26
		Hantzschia	0	5	6	8	2
		*Navicula**	0	2	0	0	0
		Total	53	41	43	39	28
	B (3.5 m)	*Pinnularia*	0	0	0	3	2
		*Nostoc***	5	0	0	0	0
		Total	5	0	0	3	2
	C (6.5 m)	Algal cell number less than 1 cell/mm²					
Site 2	D (0.3 m)	*Actinotaenium*	43	294	131		
		Pinnularia	74	14	29		
		Hantzschia	8	10	22		
		Navicula	8	0	2		
		Nostoc	0	0	5		
		Total	133	318	189		
	E (1.6 m)	*Pinnularia*	0	0	0	0	2
		Navicula	0	0	0	2	0
		Total	0	0	0	2	2
	F (2.9 m)	Algal cell number less than 1 cell/mm²					
	G (4.2 m)	〃					
Site 3	H (0 m)	*Actinotaenium*	51	59	280	110	10
		Pinnularia	267	155	245	209	160
		Hantzschia	2	0	0	0	2
		Nostoc	0	0	30	2	0
		Total	320	214	555	321	172
	I (1.5 m)	Algal cell number less than 1 cell/mm²					
	J (3.0 m)	Algae not detected from the glass slide					
	K (4.5 m)	〃					

* *Navicula muticopsis*, ***Nostoc* sp.

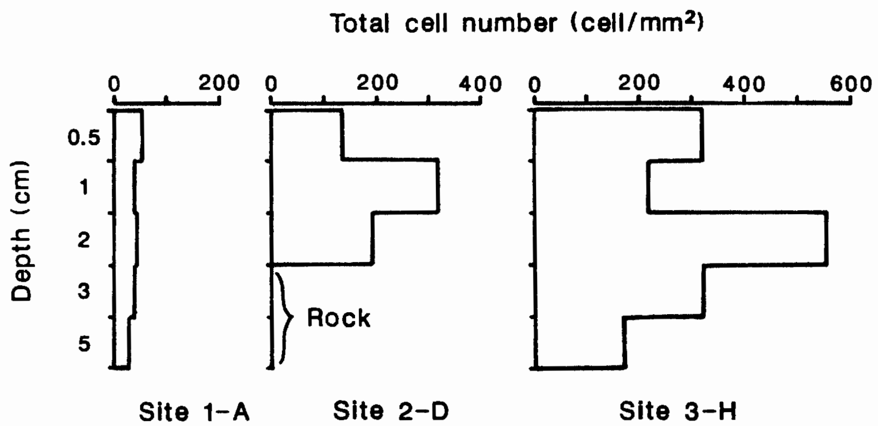

Fig. 5. Vertical change of algal cell density (cells/mm²) at three transect points along the side of the stream in the Yukidori Valley.

able tendencies of the vertical distribution of each species, however, were not found.

Among the 11 transect points at sites 1, 2 and 3, glass slides of site 2-E, site 3-I, and site 3-H were lifted 3 mm, 3 mm and 10 mm, respectively, by the freeze and thaw cycle of soils. As a result, much more light probably got through these glass slides compared with other sites where no rise of glass slides was occurred. The cell number of soil algae might be enhanced by much light and the vertical distribution of the soil algae shifted upward at these 3 transect points. The cell number at site 2-E and at site 3-I was less than 2 cell/mm^2. The enhancement of cell number by the light was, however, quite low at these two sites.

4. Discussion

All species of soil algae identified into the specific level in the present study have been reported from the moss colonies (OHTANI, 1986) and/or from the stream and pond (HIRANO, 1979) in the Yukidori Valley. Species composition of soil algae on the glass slide is especially similar to that of moss colonies (OHTANI, 1986). Species which dominantly occurred on the glass slide such as *Actinotaenium cucurbita*, *Pinnularia borealis* were also dominant on the moss colonies, though, the amount of *Nostoc* in the soil was small compared with that of the moss colonies. HIRANO (1979) reported 43 taxa of blue-green algae and 22 taxa of green algae from the stream in the Yukidori Valley, which included all the species of blue-green algae reported in the present study and *Actinotaenium cucurbita*. Diatoms of *Hantzschia amphioxys*, *Navicula muticopsis* and *Pinnularia borealis* from soils were often reported from ponds and streams in the vicinity of Syowa Station (*e.g.* FUKUSHIMA *et al.*, 1973). The species of the soil algae reported in this paper occurred in a wide range of habitat such as on moss colonies, in soils, ponds and streams.

By the direct observation of soil particles using the SEM, CAMERON and DEVANEY (1970) showed that oscillatorioid blue green algae rapidly secrete sheath material and that the sheath binds microorganisms and soil particle. Our observation by the SEM of the surface of the glass slide indicated that a filamentous blue-green alga, *Lyngbya martensiana* secreted much mucilaginous substance in a year. Its mucilages may play an important role in binding soil particles (Fig. 4: 1, 2). The direct observation of soil particle by the SEM requires another slide specimen for the identification by the light transmission microscope. It is difficult to observe exactly the same point by the SEM and the light microscope. However, using the contact slide method, one can observe exactly the same point by both the SEM and light transmission microscope. The method is useful for both the identification and the detail observation of surface feature of the algal cells.

The water availability has been considered to be one of the most crucial factors in limiting the distribution of terrestrial Antarctic algae. AKIYAMA *et al.* (1986) showed a positive correlation between the water content and the chlorophyll content in the Antarctic soil near Syowa Station. In the present study, the algal cell number and species number showed a steep decrease at only a few meters distance from the stream. Our result also showed the importance of available water in soils for the distribution of the soil algae. HOSHIAI and MATSUDA (1979) pointed out that nutrients also influenced the algal distribution near penguin rookeries. AKIYAMA *et al.* (1986) showed that nitrate concentration was correlated positively with both the water content and the chlorophyll content. In the Yukidori Valley, excrements from snow petrels nests on the cliff might be dissolved into meltwater of snow. Nutrients may also influence on the distribution of the soil algae in the Yukidori Valley.

In the present study, glass slides were vertically buried so as to make their surface portion correspond to the surface of soils or moss colonies. Therefore, the light penetrated into the deeper part of the soil through the top surface of glass slides than a real depth in the field condition. The penetrated light might encourage algal growth. The resting cells stored in the deep part of soils may grow on the glass slide along with the living cells. Our results might show the potential of the cell number of soil algae in the field.

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