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EXPERIMENTAL ARTICLES

Interaction of Cyanobacteria with Volcanic Ashes

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Abstract—Capacity for growth in water suspensions of volcanic ashes was shown for two oscillatorian cyanobacterial isolates from different environments. Growth dynamics depended on the physicochemical characteristics of the ashes and on pH of the medium. During cyanobacterial growth, some elements were leached, which either stimulated or inhibited growth. These solubilized elements could be adsorbed on the mucous sheaths, mineralizing the trichomes. The extracellular polysaccharides excreted by cyanobacteria facilitated adhesion between the ash particles and the changes in their composition. These results suggest an analogy between the processes in the modern volcanogenic areas and the biological weathering on volcanic soils during the early period of life on Earth.

Keywords: volcanic ashes, cyanobacteria, leaching, mineralization

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Biogenic weathering of rocks and minerals caused by various microorganisms and the products of their metabolism is presently a subject of intense investigation [1, 2]. Research on volcanic ashes is of special interest, since it deals with the matter arriving into the biosphere directly from the Earth interior and acting as the initial material for volcanogenic sedimentary lithogenesis and soil formation.

The publications on the interaction of microorganisms with volcanic ashes are scarce. Brock stated the fact of microbial overgrowth on ashes [3]. Pimenov described the diversity of microorganisms developing on ashes [4]. Kuzyakina investigated the ashes collected during eruptions of several volcanoes: Tyatya (Kunashir, Kuril Islands), 1973; Tolbanchik (Kamchatka), big fracture eruption, Northern breach, 1975; Alaid (Atlasov Isle, Kuril Islands), 1981; and Bezymyannyi (Kamchatka), 1980 [5]. The author demonstrated that bacteria were practically absent from the samples of fresh ashes (the ashes were sterile). Experiments on inoculation of ashes with bacteria showed no growth on sterile and nonsterile ashes. The author suggested that fresh volcanic ashes were unfavorable for microorganisms due to the presence of toxic compounds [5]. However, Karpov et al. described active development of cyanobacterial mats in Lake Karymskoe resulting from the changes in the water composition caused by an abundant ash fall during the eruption of the Karymskii volcano [6].

Experimental investigation involving anoxygenic phototrophic bacteria (APB) revealed the stimulatory effect of volcanic ashes on their growth [7]. The products of bacterial metabolism formed organo-mineral complexes with the ash substrate. This was probably the initial stage of formation of clay minerals from volcanic ashes.

The ashes are low in nutrients and energy sources. Among the microorganisms arriving onto the ashes from air, with atmospheric precipitation, and during snow melting, only those with economic metabolism and the adaptation to nutrient-poor environments may survive. Such are, for example, members of the ecologo-trophical group of oligotrophic and facultatively oligotrophic microorganisms [4, 5]. Cyanobacteria, being photosynthetic and nitrogen-fixing microorganisms capable of growth under extreme conditions, should also be expected to participate in the transformation of volcanic ashes.

The goal of the present article was to determine whether volcanic ashes are a substrate favorable for cyanobacterial growth, whether cyanobacteria participate in the transformation of ash, and whether mineralization of cyanobacteria occurs in the presence of ash.

MATERIALS AND METHODS

Algologically pure cultures of cyanobacteria from the collection of the Laboratory of Relict Microbial Communities, Institute of Microbiology, Russian

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Table 1. Macroelement content in volcanic ashes, %

Oxides	5251 Ash no. 1	5203 Ash no. 2	4495 Ash no. 3
SiO_2	59.95	63.13	62.66
TiO_2	0.85	0.96	0.98
Al_2O_3	15.36	15.47	16.42
Fe_2O_3	6.53	7.67	1.84
FeO	–	–	4.14
MgO	0.79	0.88	0.15
CaO	6.32	6.41	2.11
Na_2O	–	–	4.89
K_2O	1.43	1.53	4.27
TiO_2	0.85	0.96	–
P_2O_5	0.052	0.059	0.21
MnO_2	0.125	0.136	0.13

Note: "–" stands for not determined.

Table 2. Microelement content in volcanic ashes, μ/kg

Elements	5251 Ash no. 1	5203 Ash no. 2	4495 Ash no. 3
Ni	9	434	25.4
Cu	38	95	49.2
Zn	81	102	59
Ga	23	20	9
As	–	–	16.1
Cd	–	–	0.19
Pb	14	5	28.6
Rb	26	26	28.6
Sr	432	415	210
Y	30	31	–
Zr	165	176	–
Sn	–	–	0.82
Cs	–	–	31.6
Sb	–	–	13.8

Note: "–" stands for not detected.

Academy of Sciences were used in this work. The cultures originated from two different environments: *Oscillatoria terebriformis* was isolated from Uzon hydrotherms (Kamchatka) and grew at pH 7.8 (neutrophilic), while *Phormidium* sp. from the Khilganta soda lake (Buryat Republic) grew at pH 10 (alkaliphilic). Cyanobacteria were grown in optimal media. After five days, the biomass was collected on a plankton net, washed three times with water, and homogenized in a porcelain mortar. The thick suspension (5 mL) was added to the vials with 50 mL of the medium or of sterile deionized water supplemented with ash (1 g). The cultivation was carried out at 28°C under illumination (2000 lx).

The experiment was carried out in four variants: (1) cyanobacteria in optimal media (Zavarzin medium [8] for *O. terebriformis* and M medium [9] for *Phormidium* sp.); (2) cyanobacteria in distilled water supplemented with a known amount of powdered ash; (3) cyanobacteria in distilled water without ash; and (4) distilled water with ash not inoculated with cyanobacteria. To provide for growth of the alkaliphilic strain, distilled water (variants 2, 3, and 4) was supplemented with 3 g/L $NaHCO_3$, 17 g/L Na_2CO_3, and 30 g/L NaCl. The results were determined after 0.5, 1.5, 2, and 3.5 months. For analysis of growth dynamics, additional samples were collected after 10 and 20 days of incubation.

Cyanobacterial growth was assessed visually (as the rate of spreading in petri dishes on liquid medium) and microscopically. Since increase in dry biomass was impossible to determine in the presence of fine ash particles not separable by centrifugation, the growth rate was determined spectrophotometrically by the relative content of chlorophyll *a* (KFK-3, Russia, 665 nm). The content of a vial with ash and cyanobacteria was shaken and the liquid transferred into a petri dish. The filamentous cyanobacteria, which had a tendency for biofilm formation, remained in the vial. This biomass was extracted with 10 mL of 80% ethanol for 24 h, and OD_{665} of the extract was determined. The ash-containing precipitate was washed three times with distilled water, centrifuged, and dried. The changes in the elemental composition of the ashes after their interaction with cyanobacteria were determined relative to the ashes incubated under the same conditions without cyanobacteria.

The change in pH during the experiment were monitored using an Expert 001 pH-meter-ionomer (Russia). Microscopy was carried out on a CamScan-4 scanning electron microscope (Cambridge, United Kingdom) equipped with a Link-860 analyzer.

The ash samples were collected by G.A. Karpov immediately after eruption of the Kamchatka volcanoes Karymskii in 2008 (ash 1, sample no. 5251) and 2003 (ash 3, sample no. 4495) and Bezymyannyi in 2006 (ash 2, sample no. 5203). The samples differed in chemical composition and content of macro- and microelements (Tables 1 and 2). These data were obtained by X-ray fluorescence analysis of the samples in the Analytical center, Institute of Volcanology and Seismology, Russian Academy of Sciences, Far Eastern Branch by N.I. Chebrova, V.M. Ragulina, and V.V. Dunin-Barkovskaya. To prepare aqueous extracts, 100 g of ash was incubated in 1 L of water for 24 h.

The gravimetric composition of the ashes was determined as described in [10].

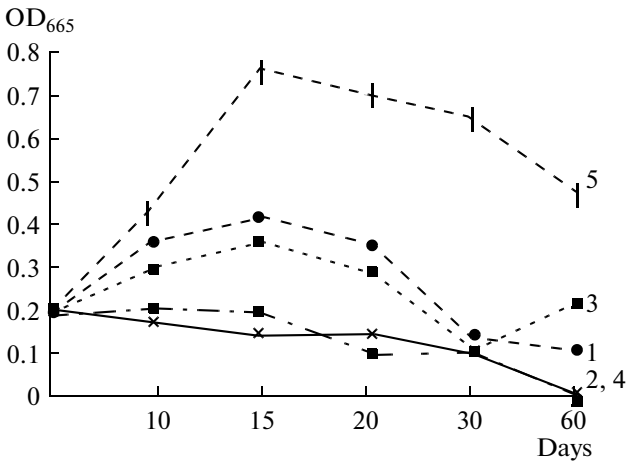

Fig. 1. Growth dynamics of *O. terebriformis* in the media with different ashes (OD_{665}): ash numbers (1–3), water (4), and medium (5).

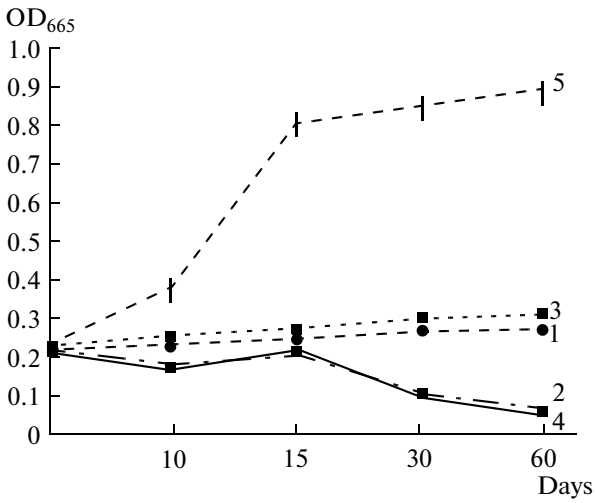

Fig. 2. Growth dynamics of *Phormidium* sp. in the media with different ashes (OD_{665}): ash numbers (1–3), water (4), and medium (5).

Chemical analyses of the net content of the elements in powdered ashes during the laboratory experiments were carried out by P.I. Kalinin at the Center for Joint Use of Scientific Equipment, Institute of Physicochemical and Biological Problems of Soil Science, Russian Academy of Sciences using a Spectroscan MAX-GV spectrometer.

The results presented are averages of the experiments carried out in three repeats and three parallel variants.

RESULTS AND DISCUSSION

Dynamics of cyanobacterial growth varied depending on the ash sample (Figs. 1 and 2). For example, on the 10th day, growth of *O. terebriformis* on ashes 1 and 3 was similar to that in the optimal medium, while in the case of ash 2 it was as weak as in pure water. After 15 days, growth on ashes 1 and 3 weakened, although it was still higher than on water. Growth on all ashes subsequently stopped, but the morphological picture was different on different ashes. On ash 2, the degradation of cyanobacterial filaments continued, while on ash 3 the filaments were surrounded by a mucous sheath. On ash 1, a laminar mat formed by day 20, providing for the viability and further development of some cyanobacteria. A stable community with pronounced laminar structure, containing living cyanobacterial filaments in the upper layer and mineralized interlayers inside the mat, developed in 1.5 months (Fig. 3c). In spite of the absence of growth on ash 3, cyanobacteria in one-month culture remained viable. Light microscopy revealed brightly pigmented filaments surrounded by abundant mucus. After two months, these filaments initiated a new peak of growth (Fig. 1).

Behavior of the alkaliphilic strain *Phormidium* sp. was different (Fig. 2). On ashes 1 and 3, this cyanobacterium remained viable throughout the experiment (two months). The increase in biomass was, however, quite low (three times lower than in the control). These conditions should be termed survival, rather than growth.

Cyanobacteria affected the ratio of various elements in the ash. Under neutral conditions, introduction of *Oscillatoria* resulted in insignificant changes in the elemental composition (Table 3), which differed little from the control variants without cyanobacteria (the contents of silicon and aluminum decreased by 1–2%, while variations in the levels of other elements were close to the detection limit). Introduction of alkaliphilic *Phormidium* sp. resulted in significant changes in the composition of ashes 1 and 3 compared to the controls (water + ash) (Table 4). In the presence of *Phormidium* the ashes lost aluminum (2 to 5.5%), silicon (7 to 17%), and Ca (6 to 9%).

In the presence of cyanobacteria, some macroelements of the ashes (Si, Al, Ca, K, Fe) were partially solubilized and participated in the mineralization of cyanobacteria. The process of mineralization commenced early. Two weeks after the onset of the experiment, mineralized films and mineral sheaths were already observed among the actively grown cyanobacterial cells (Fig. 3b). After 1.5 months, a laminar mat of *O. terebriformis* with mineral interlayers was well developed (Fig. 3c). After 3.5 months, *Oscillatoria* formed a detritus mass with poorly discernable filaments. The graph (Fig. 3d) indicates that it probably was also partially mineralized. The elemental composition of the mineralized filaments and of the mucous film changed during the experiment. In the 2-week experiment (Fig. 3a), the filaments contained less aluminum silicates than the ash (Fig. 4a), while the composition of the mineral sheaths (Fig. 3b) and the min-

Table 3. Concentrations of some elements in the ashes affected by *O. terebriformis*, %. Exposure time, 1 month

Ash no.	Na	Mg	K	Ca	Fe	Al	Si
Ash 1	–	−2.0 ± 0.1	+	–	–	−2.0 ± 0.02	−2.0 ± 0.03
Ash 2	–	+	+	+	+	−1.1 ± 0.01	−2.0 ± 0.05
Ash 3	–	–	–	−1.6 ± 0.01	+1.0 ± 0.01	−2.0 ± 0.015	−3.0 ± 0.23
$Control_1$	–	−0.21 ± 0.04	−0.28 ± 0.01	−0.3 ± 0.08	−0.1 ± 0.01	−0.53 ± 0.02	−0.14 ± 0.01
$Control_2$	–	+0.1 ± 0.02	+0.17 ± 0.03	−0.2 ± 0.01	–	−0.4 ± 0.015	−2.0 ± 0.08
$Control_3$	–	–	−0.12 ± 0.01	−0.4 ± 0.01	−0.1 ± 0.01	−0.38 ± 0.01	−1.8 ± 0.01

Note: "–" stands for a decrease by ≤0.1%, "+" stands for an increase by ≤0.1%. The controls are ash + water.

Table 4. Concentrations of some elements in the ashes affected by *Phormidium* sp., %. Exposure time, 1 month

Ash no.	Na	Mg	K	Ca	Fe	Al	Si
Ash 1	–	–	+	−8 ± 0.2	−1 ± 0.01	−3 ± 0.01	−12 ± 0.7
Ash 2	–	+	+	−9 ± 0.09	−1.4 ± 0.01	−5.5 ± 0.04	−7 ± 0.2
Ash 3	–	–	–	−6 ± 0.075	+3 ± 0.02	−2 ± 0.015	−17 ± 0.5
$Control_1$	–	− 1.7 ± 0.07	–	−1.6 ± 0.09	−0.3 ± 0.01	−1.2 ± 0.01	−3.94 ± 0.1
$Control_2$	–	+	+	−1.8 ± 0.1	−1 ± 0.01	−1.7 ± 0.15	−4.1 ± 0.2
$Control_3$	–	–	–	−1.3 ± 0.01	−1 ± 0.01	−1.2 ± 0.01	−4.9 ± 0.35

Note: "–" stands for a decrease by ≤0.1%, "+" stands for an increase by ≤0.1%. The controls are ash + water.

eral layer of the mat (Fig. 3c) was characterized by increased levels of K, Ca, and Fe.

Interaction of the ashes with *Phormidium* sp. culture also resulted in significant changes (Fig. 4). The filament morphology of the 2-week culture remained normal. The trichomes surrounding the ash particles, with the extracellular polysaccharides forming mucous films around the ash aggregates, are presented on Fig. 4c. Deformation of the cells was observed later, accompanied by formation of abundant mucus and thick mucous sheaths around the trichomes (Fig. 4d). The elemental composition of the mineral tubes was close to that of the original powdered ash (Fig. 4a). The interaction of *Phormidium* cells with ash 3 resulted in formation of a laminar, mucus-rich mat in 2 months. Its upper surface was also mineralized (Fig. 4e); the ash was decomposed (Fig. 4f). Unlike the ash (Fig. 4b), the elemental composition of the mat was characterized by emergence of Na and Cl and an insignificant increase in Ca and Fe.

Capacity of cyanobacteria for growth on ashes and the absence of growth in distilled water suggest leaching and solubilization of some elements required for microbial growth in the course of cyanobacterial interaction with ashes. In spite of small differences in the macroelement composition of the solid phase of different ashes, they had different effect on cyanobacterial growth. Growth on ash 2 was different from growth on other ashes probably due to the differences in particle sizes and the ratio of their fractions. In ash 2, the particle fraction of 0.5 mm and higher predominated (43.6%). Other fractions had particle sizes of 0.15–0.5 mm (11.7%), 0.16–3.15 mm (18%), and <0.16 mm (26.6%). Ash 1 did not contain the particles larger than 0.5 mm. The main fractions were 0.16–0.315 mm (46%), ≤0.315 mm (15.4%), 0.06–0.16 mm (23.2%), and <0.06 mm (15.4%).

Acidity of the ashes probably also affected cyanobacterial growth (aqueous extract of ash 2 had pH 4.7, while the extracts of ashes 1 and 3 had pH 5.7). Laboratory experiments demonstrated, however, that the pH decrease resulting from the introduction of acidic ashes could have no perceptible effect on cyanobacteria. In *Oscillatoria* cultures on ash 2, pH decreased from 7.8 to 6.7 and remained almost stable until the end of the experiment (pH 7.0 after one month). On ashes 1 and 3, pH decreased to 7.0 and increased to 9 during cyanobacterial growth. Addition of the ashes to the medium with *Phormidium* (pH 10) resulted in a brief insignificant pH decrease due to the buffer capacity of this solution.

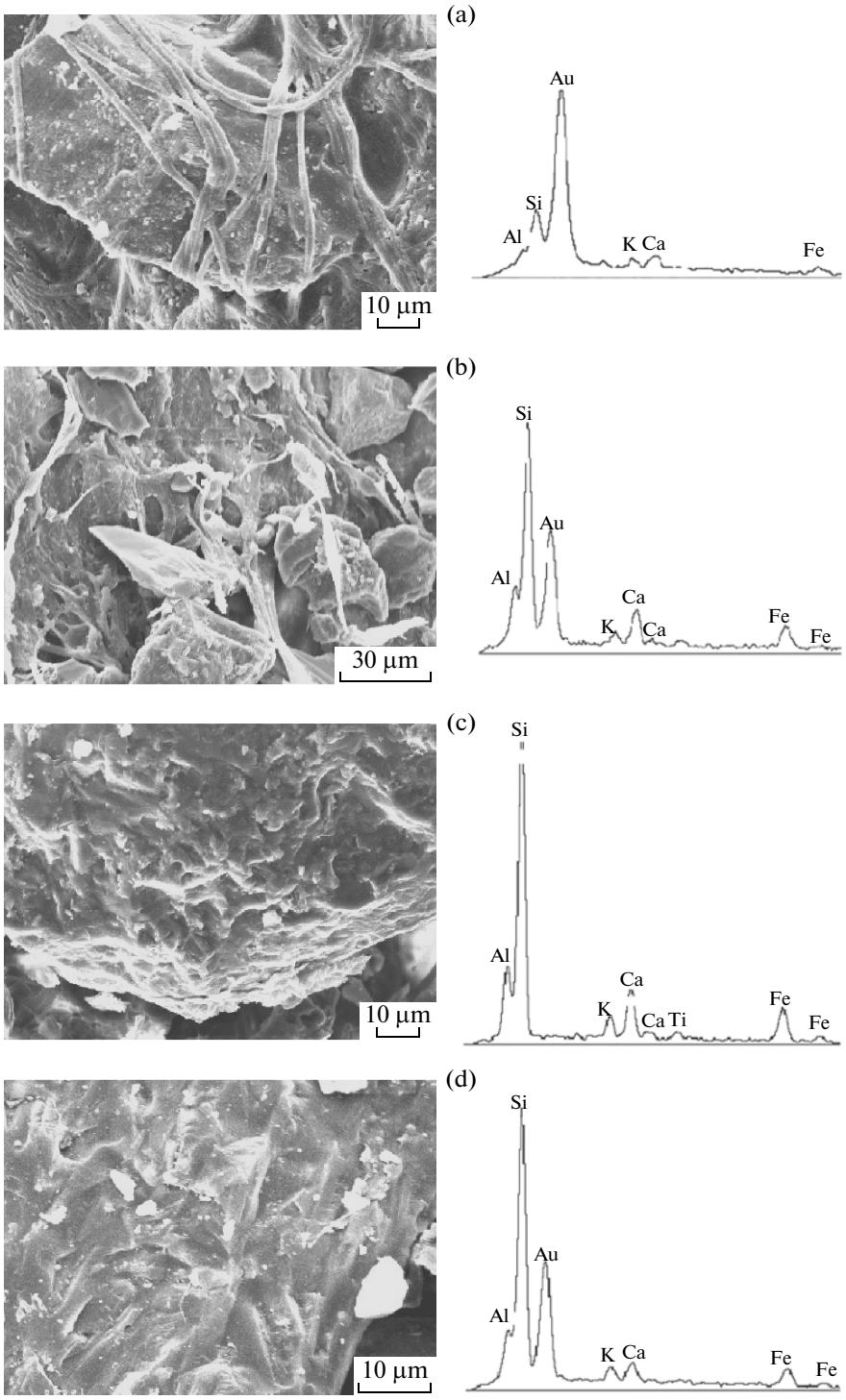

Fig. 3. Changes in morphology and elemental composition (EC) of the cyanobacterium *O. terebriformis* in the course of mineralization during growth with ash 1 at different stages of the interaction: living cyanobacterial filaments on ash, 2 weeks, and EC of the filaments (a); mineralized sheath (in the center), 2 weeks, and EC of the sheath (b); laminar mat with the mineralized upper layer, 1.5 months, and EC of the mineral layer (c); mineralized detritus mass with poorly discernible individual filaments, 3.5 monts, and EC of the detritus mass (d).

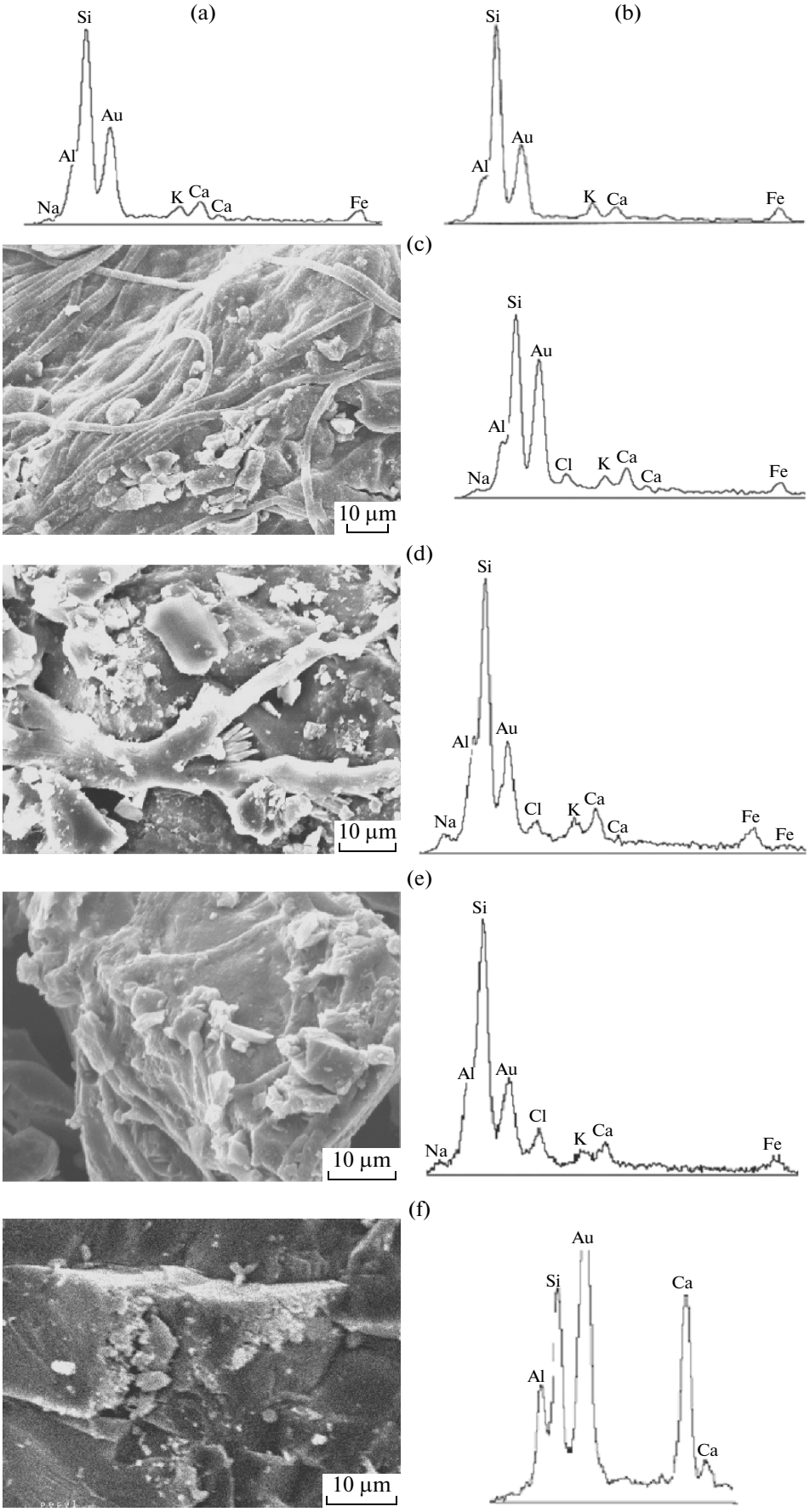
(a)
Si
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Fig. 4. Changes in morphology and elemental composition (EC) of the cyanobacterium *Phormidium* sp. in the course of mineralization during growth with different ashes: EC of ashes 1 (a) and 3 (b); live *Phormidium* filaments, partially covered with mucus with adsorbed ash particles, grown on ash 1 for 2 weeks, and EC of the trichome (c); trichomes in mineralized tubes, grown on ash 1 for 2 weeks, and EC of the mineralized tubes (d); mineralized laminar mat grown on ash 3 for 2 months and EC of the upper mat layer (e); and decomposition of ash 1 by *Phormidium* sp. after 2 months (f).

The microelement composition is the third feature differentiating the ashes. The absence of growth on ash 2 resulted probably from the inhibitory effect of heavy metals (nickel, zinc, and copper), which were present in higher concentrations than in other ashes. The inhibitory effect of ash 2 was shown in laboratory experiments with anoxygenic phototrophic bacteria, when solubilization of copper inhibited bacterial growth [7].

The differences in the effect of microorganisms on the elements of the ashes are of some interest. In the case of APB growth, "the exchange cations of calcium and sodium were actively substituted by potassium and magnesium, which probably arrived from the medium" [7]. This was not observed for cyanobacteria. The differences in the interaction of APB and cyanobacteria with ashes may be explained by different cultivation conditions, such as the presence and absence of oxygen and different growth media. Heavy metals are known to bind to different sites of the cell envelope (cell wall and mucous sheath), so that their inhibitory effect is different for different microorganisms [11, 12].

Thus, the fine grain structure of ashes 1 and 3, together with a less pronounced effect of heavy metals, were probably the factors responsible for colonization of these ashes by *Oscillatoriales* cyanobacteria.

The behavior of neutrophilic and alkaliphilic cyanobacteria growing on ashes was different, indicating that the conditions of cyanobacterial growth and development depended on the ambient pH. Alkaliphilic cyanobacteria caused more active solubilization of the elements present in powdered ashes (Tables 3 and 4). Such elements as Si, Al, and Ca were leached. In the solution, they participated in the mineralization of the cell envelope and mucus, forming a protective shell, which was responsible for cell preservation throughout the experiment (2 months). While some trichomes remained sealed in this shell, some others were able to crawl out of the mineral sheath, causing a low increase in the biomass of the alkaliphilic culture (Fig. 2).

Introduction of the neutrophilic *Oscillatoria* to the water with ash resulted in slower leaching of the elements, so that they were completely consumed during the rapid growth of the first two weeks. This nutrient limitation resulted in cessation of growth, while the inhibitory action of heavy metals intensified, causing the death of the culture. Thus, the environmental factor (pH in our experiments) plays an important role in cyanobacterial growth on fresh ashes.

The ability of cyanobacteria to recover the elements required for growth from volcanic ashes was demonstrated. This process was shown to affect the composition of the ashes. The range of leached elements depended on the chemical and physical composition of the ashes and on the environment for the growing cyanobacteria. In the presence of ashes, cyanobacteria formed a pronounced glycocalix layer on and between the trichomes, which was subsequently mineralized. Capacity of cyanobacteria for production of extracellular polysaccharides binding many metals, including the toxic ones, has a certain effect on the preservation of their viability [11]. Capacity of filamentous cyanobacteria for motion and formation of the mucous glycocalix, which cements the ash particles, results in formation of cyanobacterial mats. Since the cultures used in our experiments were not axenic, bacterial satellites were certainly also involved in this process. However, since no mats were formed in the dark, when cyanobacterial growth was absent, cyanobacteria played the major role in this process. These examples may be considered the early stage of cyanobacteria-dependent biogenic degradation of volcanic ashes and provide the basis for comparison of the processes occurring presently in volcanogenic areas and the processes of biogenic weathering of volcanic soils during the initial period of life development on Earth.

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REFERENCES

1. Karavaiko, G.I., Microbial Destruction of Silicate Minerals, in *Trudy Instituta Mikrobiologii im. S.N. Vinogradskogo* (Proc. Winogradsky Inst. Microbiol.), Gal'chenko, V.F., Ed., Moscow: Nauka, 2004, pp. 172–196.
2. *Kal'dernye mikroorganizmy* (Caldera Microorganisms), Zavarzin, G.A., Ed., Moscow: Nauka, 2004.
3. Brock, T.D., *Thermophilic Microorganisms and Life at High Themperatures*, Heidelberg: Springer, 1978.
4. Pimenov, E.P., Functioning of Microbial Complexes in the Zones of Intense Volcanic Ash Falls, *Extended Abstract of Cand. Sci. (Biol) Dissertation*, Alma-Ata: Inst. Microbiol. Virusol. KazSSR Acad. Sci., 1983.

5. Kuzyakina, T.I., Microbial Transformation of Volcanic Ash, in *Vulkanizm i svyazannye s nim protsessy* (Volcanism and Associated Processes), Petropavlovsk-Kamchatskii: Dal'nauka, 1985, no. 3, pp. 232–234.
6. Karpov, G.A., Nikolaeva, A.G., Lupikina, E.G., Bortnikova, S.B., and Ushakov, S.V., Hydrochemical and Geochemical Composition of the Matter in the Lake Karymskoe Basin in the Post-Catastrophe Period (1996–2005), *Mat. Nauch.-Tekh. Konf. "Geofiz. monitoring Kamchatki* (Proc. Sci.-Tech. Conf. Geophisical Monitoring of Kamchatka), 2007, pp. 17–18.
7. Naimark, E.B., Kompantseva, E.I., and Komova, A.V., Interaction between Anoxygenic Phototrophic Bacteria of the Genus *Rhodovulum* and Volcanic Ash, *Microbiology*, 2009, vol. 78, no. 6, pp. 747–756.
8. Orleanskii, V.K. and Gerasimenko, L.M., Laboratory Simulation of a Thermal Cyanobacterial Community, *Microbiology*, 1982, vol. 51, no. 4, pp. 538–542.
9. Gerasimenko, L.M., Dubinin, A.V., and Zavarzin, G.A., Alkaliphilic Cyanobacteria from Soda Lakes of Tuva and Their Ecophysiology, *Microbiology*, 1996, vol. 65, no. 6, pp. 736–740.
10. Shein, E.V., *Fizika Pochv* (Physics of Soils), Moscow: Mos. Gos. Univ., 2005.
11. Obst, M., Dynes, J.J., Lawrence, J.R., Swerhone, G.D.W., Benzerara, K., Karunakaran, C., Kaznatcheev, K., Tyliszczak, T., and Hitchcock, A.P., Precipitation of Amorphous $CaCO_3$ (Aragonite-Like) by Cyanobacteria: A STXM Study of the Influence of EPS on the Nucleation Process, *Geochim. Cosmochim. Acta*, 2009, vol. 73, pp. 4180–4198.
12. Nies, D.H. and Silver, S., Metal Ion Uptake by a Plasmid-Free Metal-Sensitive *Alcaligenes eutrophus* Strain, *J. Bacteriol.*, 1989, vol. 171, pp. 896–900.