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# Degradative crystal–chemical transformations of clay minerals under the influence of cyanobacterium-actinomycetal symbiotic associations

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## Abstract

Cyanobacteria and actinomycetes are essential components of soil microbial community and play an active role in ash elements leaching from minerals of the parent rock. Content and composition of clay minerals in soil determine the sorption properties of the soil horizons, water-holding capacity of the soil, stickiness, plasticity, etc. The transformative effect of cyanobacterial–actinomycetes associations on the structure of clay minerals – kaolinite, vermiculite, montmorillonite, biotite and muscovite – was observed, with the greatest structural lattice transformation revealed under the influence of association in comparison with monocultures of cyanobacterium and actinomycete. The range of the transformative effect depended both on the type of biota (component composition of association) and on the crystal–chemical parameters of the mineral itself (trioctahedral mica – biotite, was more prone to microbial degradation than the dioctahedral – muscovite). The formation of the swelling phase – the product of biotite transformation into the mica–vermicullite mixed-layered formation was revealed as a result of association cultivation. Crystal chemical transformation of vermiculite was accompanied by the removal of potassium (K), magnesium (Mg) and aluminum (Al) from the crystal lattice. The study of such prokaryotic communities existed even in the early stages of the Earth's history helps to understand the causes and nature of the transformations undergone by the atmosphere, hydrosphere and lithosphere of the planet.contribution of treatments on structure induces and model parameters are discussed in the paper.

## Article Info

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## Introduction

Microorganisms have a very diverse range of mechanisms of mineral biodegradation. They are: acid and alkali formation, biosorption, chelation (complex formation), etc. (Ivanova et al., 2012). One of the manners of bacterial leaching is the ability of exopolysaccharide release, in particular, mucus formation (Aristovskaya, 1980). The ability for slime formation is peculiar for many species of bacteria, including cyanobacteria.

Cyanobacteria and actinomycetes are obligate components of the associations of soil microorganisms in the areas of initial soil formation, soil “eutrification” as well as hydrotherms and lagoons (Andreyuk and Kopteva, 1990, Zvyagintsev et al., 2001). They participate in many biochemical processes (organic matter accumulation, mineral substrate destruction, the redistribution and accumulation of various elements, etc.) and, thus, transform the medium and take an essential part in the soil formation (Domracheva, 2005). The pedogenetic activity of modern cyanobacterial associations manifested by the formation of fouling films on

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bedrock surfaces and by the transformation of the mineral soil matrix was noted by many investigators (Budel et al., 2004; Chizhikova et al., 2005).

Preferential development of cyanobacteria is confined to specific places of weakened competition: to the outputs of the rocks, carbonate releases, etc. Colonization of silicate surface by the alkaliphylic cyanobacterial community results in the removal of exchangeable cations and leads to dissolution of the mineral substrate (Alekseeva et al., 2009). The occurrence of fossilized cyanobacterial filaments and actinomycetal hyphae indicates that communities of these organisms were the most important factor of sedimentation on the surface of the earth since the Archean (Rozanov, 2003).

Actinomycetes can act as active biodestructors mainly due to the synthesis of various hydrolytic enzymes, as well as antibiotics, vitamins, and other products of secondary metabolism. Actinomycetes are a component of the association of microorganisms (with a predominance of cyanobacteria) in cycads (Zenova et al, 2005). However, the functional potential of cyanobacteria–actinomycetal associations in natural communities has yet to be studied.

Our work was aimed at analyzing the structural alteration of clay minerals under the influence of experimental associations of cyanobacteria and actinomycetesn, and aggregate and structure stability of soils using the high energy moisture characteristic (HEMC) method.

## Material and Methods

Mineral substrates for associations' growth were rock samples of fine-dust (1-10 mkm) and fine-sand (25-50 mkm) fractions. Rocks of fine-dust were: (a) kaolin with kaolinite ($Al_4(OH)_8[Si_4O_10]$) as a predominant mineral, (b) vermiculite composed of 56% vermiculite ($((Ca, Mg, ...)(Mg, Fe)_3(OH)_2[(Si, Al)_4O_{10}] \bullet 4H_2O$), and (c) gumbrin composed of montmorillonite (Ca, Mg, …), $(Al, Fe^{3+}, Mg)_2(OH)_2[(Si, Al)_4O_{10}] \bullet nH_2O$. Two investigated rocks of fine-sand dimensions were monomineral and comprised of biotite ($K(Mg, Fe)_3[AlSi_3O_{10}](OH, F)_2$) and vermiculite ($Mg_x(H_2O)_4\{Mg_3\text{-}x[AlSi_3O_{10}](OH)_2\}$). Mineral samples were provided by the Museum of the Soil Physics and Soil Reclamation Department of the Soil Science Faculty of Moscow State University and by the Museum of V.V. Dokuchaev Soil Science Institute.

Experimental two-component associations consisted of the following cultures: free-living cyanobacterium *Anabaena variabilis* ATCC 29413 and actinomycete *Streptomyces pluricolorescens* FR837629, and another one composed from the abovementioned cyanobacterium and *S. cyaneofuscatus* strain FR837630. Actinomycetes were isolated from roots of tropical cycad plants *Cycas micholitzii* and *Stangeria eriopus* (State Botanical Garden of the Russian Academy of Sciences, Moscow). The phylogeny of actinomycetes was determined via 16S rRNA gene sequencing. Aksenic culture of cyanobacterium *Anabaena variabilis* ATCC 29413 was obtained from the museum of the Department of Physiology of Microorganisms of the Biological Faculty, Moscow State University.

Streptomycetes were grown on mineral agar_1 (G1) medium of the following composition (g/l): soluble starch, 20.0; $K_2HPO_4$, 0.5; $MgSO_4$, 0.5; $KNO_3$, 1.0; NaCl, 0.5; $FeSO_4$, 0.01; and agar, 20.0 (Gause et al., 1983). The growth of cyanobacteria A. variabilis was performed in a luminosity room on medium BG_11 of the following composition (g/l): $NaNO_3$, 1.5; $K_2PO_4$, 0.04; $MgSO_4 \bullet 7H_2O$, 0.075; $CaCl_2 \bullet 2H_2O$, 0.036; citric acid, 0.006; ammonium citric iron, 0.006; EDTA, 0.0011; $Na_2CO_3$, 0.02; and microelements (1 ml). Cyanobacterial monocultures were reinoculated once per month; the volume of cyanobacterial inoculates was equal to 10% of the fresh medium volume. To obtain mixed cultures, cyanobacteria were grown in a liquid medium BG_11 under the illumination of 780 lx and 24 ± 1°C.

The experimental cyanobacterial–actinomycetal associations were created from the inoculums of streptomyces cultivated in G1 medium for seven days and cyanobacteria cultivated in BG_11 medium for three weeks under conditions of permanent light (780 lx, 24 ± 1°C). The components were mixed together (the initial biomass ratio was 1:1) and grown in a modified liquid medium BG_11 under the illumination for seven–ten days. The modification of the medium consisted of the absence of microelements in it. The use of the medium traditionally applied to cultivate cyanobacteria, but without microelements, was based on the assumption that an experimental association of microorganisms may grow successfully under conditions ensuring their growth, but not optimal for individual components of the association.

Experiments were conducted in glass beakers with sealed pore membranes barrier filter and 5 mm layer of the mineral base were placed upon the membrane. The biomass of monocultures of cyanobacterium,

actinomycetes and associative thallus (about 1.8-2 g) were placed on the mineral surface. The experiments on bacterial leaching of crystal lattice of minerals investigated lasted for 2 months.

A modified medium BG_11 was applied once per two–three days during two months to sustain the growth of the cyanobacterial–actinomycetal associations. The added medium not only ensured their growth but also washed out the products of metabolism into the underlying layers of mineral fractions.

The composition of mineral samples and the structural parameters of crystal lattice were determined by the X-ray diffractometry on a universal Carl Zeiss Jena XZG diffractometer (Germany) (Gorbunov, 1963). The operating mode of the device remained constant (30 kV, 40 mA, copper radiation, nickel filter). X-ray curves were obtained for air-dried samples, samples saturated with ethylene glycol, and samples incinerated at 550°C for 2 h. The identification of clay minerals was perfomed according to standard procedures (Gradusov, 1973; Sokolova et al., 2005).

## Results and Discussion

According to the X-ray analysis, the initial kaolin sample consisted of kaolinite with some admixtures of mica, gibbsite, quartz, and feldspar (orthoclase). Kaolinite was identified on the base of clearly pronounced peaks at 7.22 ($d_{001}$) and 3.58 Å ($d_{002}$) for air-dry samples (Figure 1 (I), curve *a*). After saturation with ethylene glycol, the peaks remained unchanged (Figure 1 (I), curve *b*). Incineration of the specimens at 550°C for two hours destroyed kaolinite (Figure 1 (I), curve *c*).

Mica in the initial sample was detected by an integer series of peaks at 10.0 Å ($d_{001}$), 5.01 Å ($d_{002}$), and 3.34 Å ($d_{003}$) (Figure 1 (I), curve *a*). After saturation with ethylene glycol and after incineration, these peaks remained unchanged (Figure 1 (I), curves *b* and *c*). The ratio between the intensities of the peaks at 10.0 and 3.34 Å to that at 5.01 Å is about 3:1, which points to the presence of the dioctahedral mica.

The presence of quartz was identified by the peaks at 4.28 and 3.34 Å. The peat at 3.22 Å belongs to a feldspar, and the peak at 4.85 Å belongs to gibbsite.

The growth of the association of actinomycete *S. cyaneofuscatus* and cyanobacteria *A. variabilis* led to some transformation of the minerals. A considerable decrease in the intensities of the peaks characteristic of phyllosilicates (primarily, mica) took place (Figure 1 (III), curve a). At the same time, the intensities of the peaks characteristic of quartz (4.28 and 3.34 Å) and gibbsite (4.85 Å) increased (Figure 1 1 (III), curve a). It can be supposed that these changes pointing to some disordering of the structure of phyllosilicates took place under the impact of the growth of the cyanobacterial–actinomycetal association.

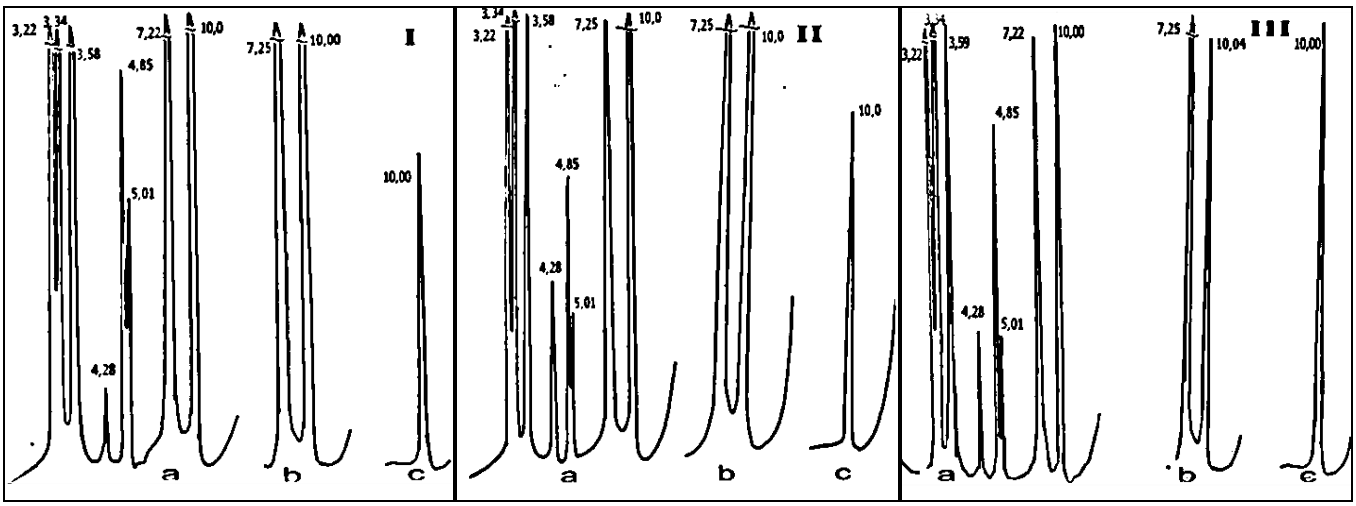


Figure 1. X-ray diffractogramms of clay minerals in the fine-dust fraction (1-10 mkm) of kaoline rock: I – initial sample; II – sample after 2-month growth of *S. pluricolorescens* monoculture, III - sample after 2-mounth growth of associative thalli. Here and in other Figs a, b, and c designate air-dry samples saturated with ethylene glycol, and samples incinerated at 550°C for 2 h, respectively.

A similar experiment was performed with the rock composed of vermiculite and biotite. Vermiculite in the initial sample was diagnosed by the presence of the high peak of the first order at 14.4 Å and a series of basal

peaks (with the highest peaks of the fourth and fifth orders) at 3.64 and 2.88 Å (Fig. 2 (I), curve a). The saturation of the sample with ethylene glycol did not alter basal interplanar distances (Fig. 2 (I), curve b). The incineration at 550°C did not result in the complete shrinking of the mineral lattice to 10.0 Å, though the intensity of the first peak (14.04 Å) decreased considerably (Figure 2 (I), curve c).

Biotite was identified from an integer series of peaks multiple of 10.2 Å (d002 = 5.07 Å and d003 = 3.38 Å) (Figure 2 (I), curve a). These peaks did not change upon the specimen saturation with ethylene glycol and incineration at 550°C for 2 h (Figure 2 (I), curves b and c). The growth of the association of actinomyces S. cyanofuscatus and cyanobacteria A. variabilis caused the transformation of these minerals. The intensities of the peaks in areas d001, d004, and d005 decreased by three times (Figure 2 (II), curve a), which points to the destruction of these minerals. The decrease in the intensities of the peaks characteristic of biotite was less significant than that of the peaks characteristic of vermiculite. However, in the case of biotite, a new swelling phase—the product of biotite transformation into a mixed-layered mica–smectitic formation—could be diagnosed by the appearance of new peaks at 12.7 and 24.4 Å (Figure 2 (II), curve a).

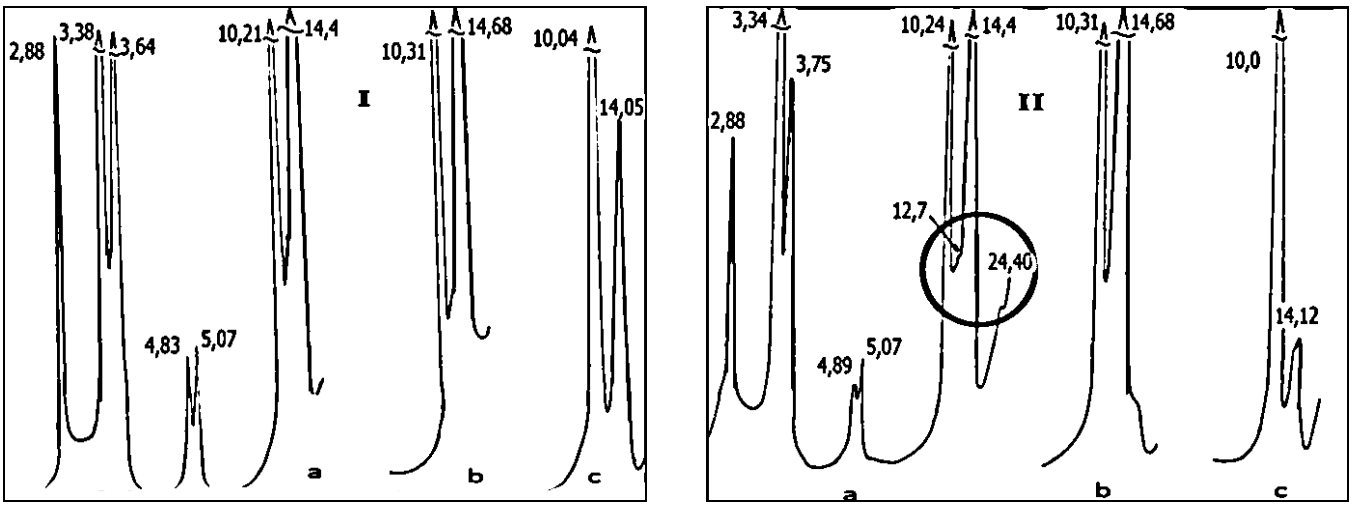


Figure 2. X-ray diffractogramms of clay minerals in the fine-dust fraction (1-10 mkm) of vermicullite rock: I – initial sample; II - sample after 2-mounth growth of associative thalli of *A. variabilis* and *S. cyaneofuscatus*.

The results obtained in the experiments with kaolin and vermiculite attest to some differences in the character of changes of these substrates under the influence of the products of metabolism of *S. cyanofuscatus* and *A. variabilis*. These differences are due to the specificity of the crystal chemistry of studied minerals and their tolerance toward weathering. The alteration of vermiculite was more pronounced than the alteration of kaolin. The amount of vermiculite minerals decreased considerably (as judged from the decrease in the intensities of major peaks). The rate of transformation of micas mixed-layered minerals depends on the mica structure: trioctahedral mica (biotite in the composition of vermiculite) is transformed considerably faster than dioctahedral mica (mica in the composition of kaolin). X-ray diffractometry of oriented slides prepared from gumbrin attests to the monomineral composition of this material; it consists of montmorillonite. In the initial air-dry state, montmorillonite is diagnosed by a distinct peak at 14 Å. The subsequent integer series of the peaks have much lower intensities (Figure 3 (I), curve a). The saturation of the sample with ethylene glycol caused an increase in the interplanar distance from 14 to 16.9 Å; a new integer series of the peaks appeared, and the peak at 8.6 Å was clearly pronounced (Figure 3 (I), curve b). Incineration of the slide at 550°C led to the shrinking of the interplanar distance to 10.0 Å. The peaks at 4.07 and 3.35 Å (curve a) attest to some admixture of cristobalite and quartz, respectively.

Gumbrin was subjected to the influence of two microbial associations: (a) *A. variabilis* and *S. pluricolorescens* and (b) *A. variabilis* and *S. cyaneofuscatus*. The use of *S. pluricolorescens* as a mycelial component caused a considerable decrease in the intensity of the main (14 Å) peak (Figure 3 (II), curve a). At the same time, an abrupt increase of the peaks at 4.4, 4.07, and 3.35 Å took place. It may be supposed that the disordering of the mineral structure along c axis took place due to the aggregation of the minerals with the products of metabolism of the microbial associations. An increasing intensity of the main peak at 4.4 Å is an argument in favor of this assumption. A relative decrease in the montmorillonite content was also confirmed by an increase in the content of quartz and cristobalite (Figure 3 (II), curve a). The saturation of the sample with ethylene glycol caused the expansion of the interplanar distance to 17.24 Å, and the incineration of the

specimen at 550°C led to its decrease to 10.0 Å (Figure 3 (II), curves b and c). The association with participation of *S. cyaneofuscatus* as a mycelial component also had an effect on the structure of the minerals. The major peak of montmorillonite (d001 = 15.2 Å) remained clearly pronounced, though less intense (Figure 3 (III), curve a). The results of X-ray diffractometry of the samples saturated with ethylene glycol and incinerated at 550°C were similar to those described for the previous sample.

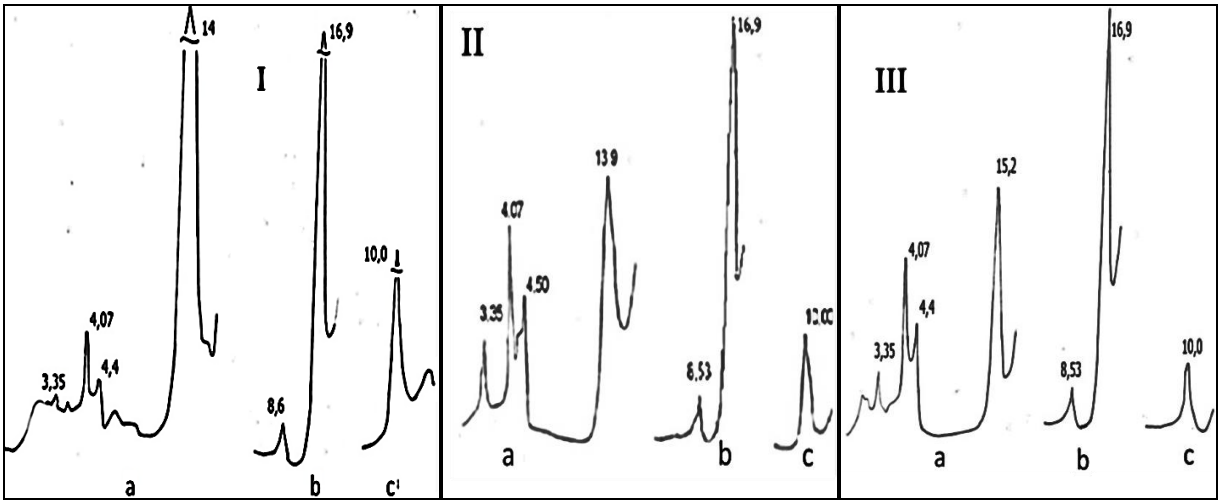


Figure 3. X-ray diffractogramms of clay minerals in the fine-dust fraction (1-10 mkm) of gumbrine: I – initial sample; II - sample after 2-mounth growth of associative thalli of *A. variabilis* and *S. cyaneofuscatus*; III - sample after 2-mounth growth of associative thalli of *A. variabilis* and *S. pluricolorescens.*

The association of cyanobacterium with *S.pluricolorescens* active in the transformation of kaolinite substrate. It was determined by the significant decreasing in the intensity of mineral reflexes in the rock sample in comparison to the sample undergo the growth of cyanobacterium-*S.cyaneofucatus* association (Figure 4, I and II).

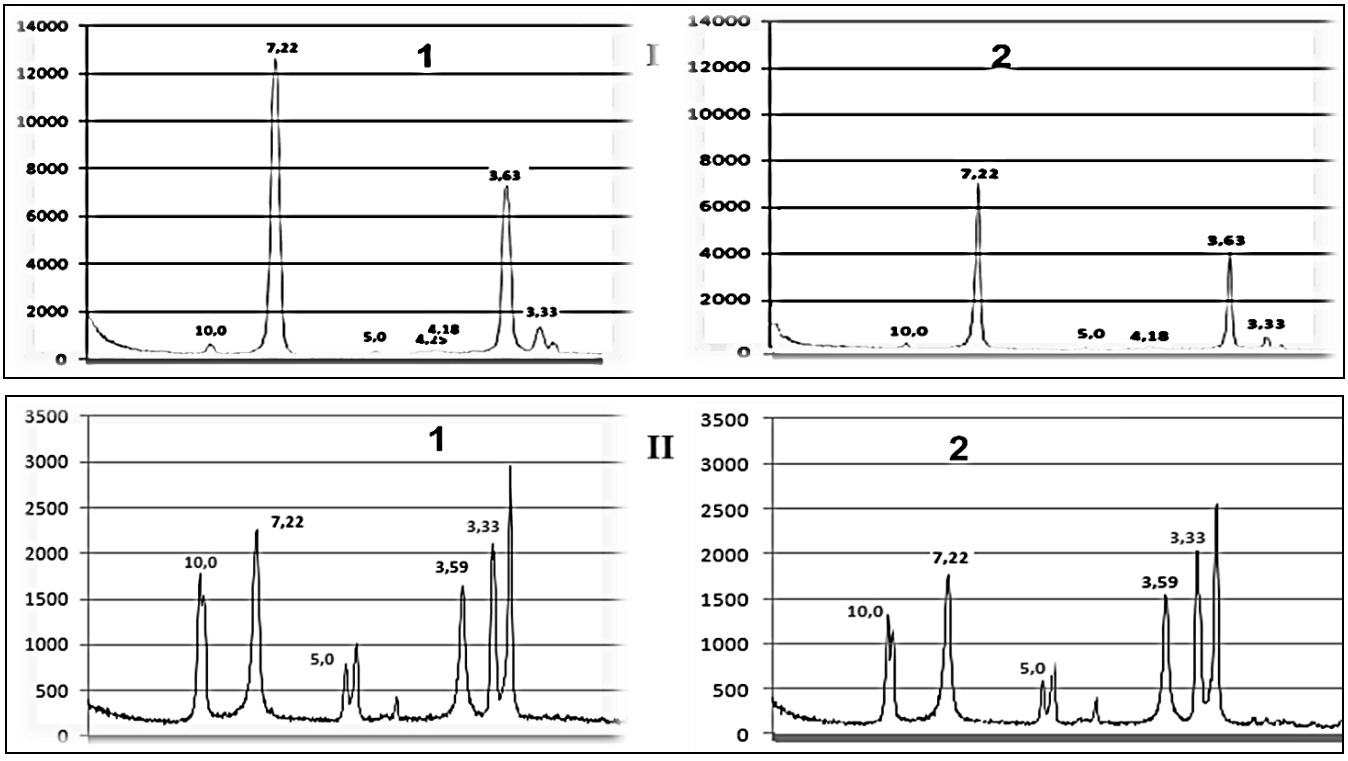


Figure 4. Comparison of X-ray diffractogramms of clay minerals of the air-dried sample of the fine-dust fraction (1-10 mkm) of kaoline rock: 1 – the initial samples, 2 – samples after the growth of experimental associative thalli: I – *A.variabilis + S.pluricolorescens*; II – *A.variabilis + S.cyaneofuscatus.*

The X-ray analysis of fine sand fraction (25-50 microns) of vermiculite and biotite indicated the significant transformation of the structure of minerals, diagnosed by the reducing in the intensity of reflexes vermiculite (Figure 5) and biotite (results no shown) as a result of the growth of cyanobacterium, streptomycetes and actinomycete-cyanobacterial associations. The sedimentation analysis of mineral substrate suspected to the influence of associative thallus indicated the occurrence of the dispersion process of the rock sample material resulting in the destruction of fine sand particles to fine-dust dimensional ones.

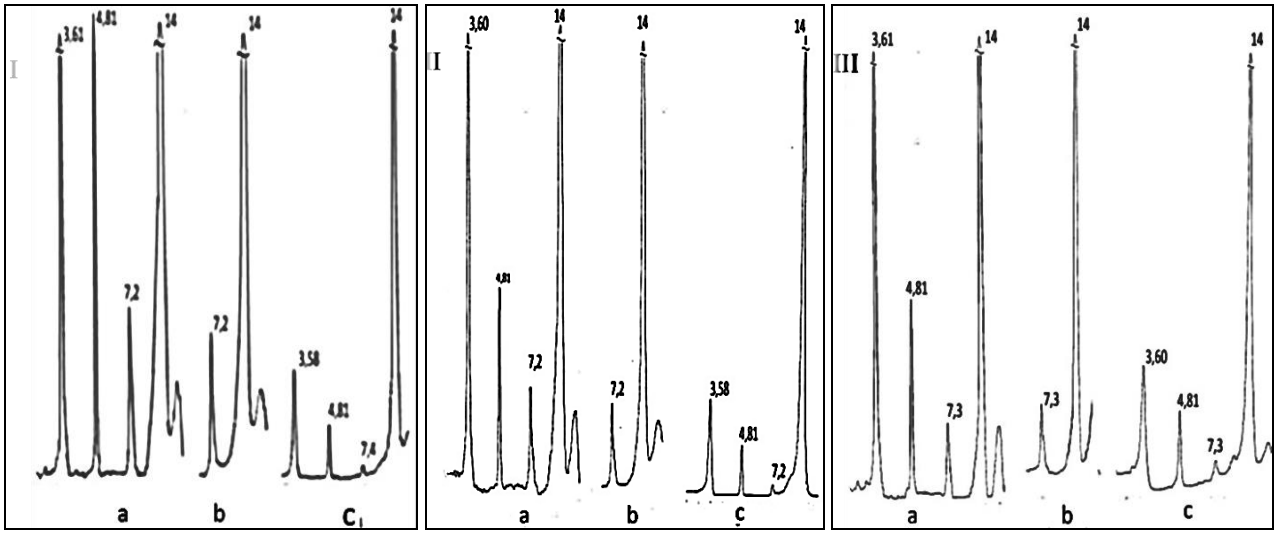


Figure 5. X-ray diffractogramms of clay minerals in the fine-sand fraction (25-50 mkm) of vermicullite rock: I – initial sample; II - sample after 2-mounth growth of A.variabilis monoculture; III - sample after 2-mounth growth of associative thalli of *A. variabilis* and *S. pluricolorescens.*

Thus, cyanobacterial-actinomycete thallus growth leads to the physical crushing of the mineral substrate, which in turn leads to a strong degradation of mineral structure. The establishment of associative experimental thallus on the mineral surface cause the chemical dissolution along with the physical crushing of vermiculite; the latter is most likely a consequence of the symbiotic nature of interaction between the components of the thallus – co-stimulation components to each other and, therefore, more complete extraction of nutrients elements from the mineral.

These data indicate the presence of process failure mineral structure, despite the significant increase in the size of the mineral particles. However, it should be noted that this model of experience is close to real environmental conditions, in particular to the situation outcrop of rock, on which the first settlers are cyanobacteria in symbiotic associations with filamentous bacteria.

The cultivation of cyanobacterial-associative aktinomitsetal thallus and monocultures of cyanobacteria and actinomycetes on vermiculite rock promoted the removal of the potassium, magnesium and aluminum - the products of mineral crystal lattice destruction. Cation leaching caused by the influence of associative thallus exceeded the same observed in the sample with monoculturing of cyanobacterium *A.variabilis*, *S. pluricolorescens* and in the control sample (Table 1). Thus, the biodegradative effect of associative thallus was more pronounced in comparison to monocultures of cyanobacterium and actinomycetes.

Table 1. Leaching of cations from the rock of vermiculite under the influence of *A. variabilis* and *S. pluricolorescens* monocultures and their associative thalli development

| | Mg | Al | K |
|---|---|---|---|
| | mg/l | | |
| S. pluricolorescens | 108,10 ± 17,27 | 0,005 ± 0,001 | 2,40 ± 0,45 |
| A.variabilis | 42,82 | ± 6,85 | 0,018 ± 0,004 |
| A.variabilis + S. pluricolorescens | 198,20 | ± 25,71 | 0,074 ± 0,011 |
| control | 17,07 | ± 1,87 | 0,007 ± 0,001 |
| $H_2O$ distilled sterilized | 0,51 ± 0,08 | 0,015 ± 0,003 | 0,03 ± 0,01 |

## Conclusion

The experimental cyanobacterial–actinomycetal associations affected the structural parameters of clay minerals (kaolinite, vermiculite, biotite and montmorillonite). The growth of experimental thalli results in the crystal-chemical alteration of the lattice of minerals of fine sand and more coarse – fine-size fractions. The greatest change of the structural parameters of the clay minerals was noted under the growth of the cyano-actinomycetal associations in comparison with the monocultures of its components. The way of the prokaryotic association influence depends both on the crystal-chemical composition of the mineral (trioctahedral micas (biotite) are more subject to microbic degradation in comparison with diocthaedral) and on the composition of the association itself.

The transformation of clay minerals under the impact of organisms proceeds in modern soils. The crystalline structure of clay minerals is subjected to some disordering under the influence of microorganisms and the products of their metabolism. This is accompanied by the release of mineral nutrients that become available to the microbial associations. One can suppose that the analogous processes of transformation of the rocks could take place in the Precambrian period under the impact of the algal–bacterial associations preserved as lithified stromatolites with an age of about 3.5 billion years during the initial stages of pedogenesis (rock transformation). As a result, new substrates were produced that were later colonized by the higher organized organisms and plants. This led to the further development of pedogenesis on the planet.

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