THE ROLE OF MICROORGANISMS IN THE WEATHERING OF ROCKS

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II. Focal Distribution of Microorganisms on the Surface of Rocks /492*

In an inspection of the overgrowth of lichens on rocks, one feature becomes very evident, i.e., that the lichens do not usually grow over the rock masses in a continuous layer, but in pockets, spots, and foci. In the chaos of piles of stones of the same type of rock (on the scarps of mountain cliffs, in quarries, and so on) it is frequently seen that some boulders or rocks are densely covered with lichens, while other neighboring ones -- no different at all in outward appearance -- remain completely clean, without a single spot or a single colony of these organisms.

In manmade structures built of stones of a single type of rock, some stones become covered with lichens and others are entirely free of growth, or else only small areas thereof and isolated slabs are covered with lichens.

We have observed this pattern of selective lichen growth on the columns in the fundament of the ancient temple in Echmiadzin (Armenia), as well as on the ruins of the ancient cathedral in Zvartnots (near Echmiadzin). In both places stones of the same rock were overgrown with lichens to a very different degree; some stones were completely free and other adjoining stones had been entirely overgrown.

The same thing may be seen on modern buildings -- houses, bridges, etc. -- both in low-lying places, in valleys, ravines, as well as on high mountain tops.

This is most distinctly apparent, however, on funerary monuments and stones. From the erection dates indicated on them, not only may the degree, but also the rate of lichen overgrowth on the rocks be exactly determined. We drew up an account of gravestones with and without lichen overgrowth in cemeteries in different regions of the city and in different settlements (Armenia) and divided the stones by the dates at which they were laid and by the rock from which they were made. The findings are given in Table I.

The investigations showed that degree and rate of overgrowth of the stones /493 depend on the nature of the rock. Basalt rocks are more solidly overgrown than are tufas (tuff rocks).

Of the 211 gravestones counted, which were erected since 1884, only 59 remained clean and untouched by lichens. This is 28%; of the 135 stones of red tuff in the same period, 75 stones or 55% remained clean. Out of the 140 stones of black tuff, 115, or 82%, retained their clean appearance.

As the quoted figures show, it is black tufa which has the least lichen overgrowth. In sixty years most of these gravestones have up until now remained

*Note: Numbers in the margin indicate pagination in original foreign text.

TABLE I.

Time of Erection of Gravestones by Type of Rock	Basalt				Red Tuff				Black Tuff			
		Overgrown				Overgrown				Overgrown		
	Tot. No. Stones	Solidly	Spottily	Not Overgrown	Tot. No. Stones	Solidly	Spottily	Not Overgrown	Tot. No. Stones	Solidly	Spottily	Not Overgrown
1884-1900	38	20	12	6	25	10	8	7	22	1	6	15
1901-1915	42	25	9	8	29	8	12	9	26	1	7	18
1916-1925	20	17	8	5	16	2	6	8	16	0	5	11
1926-1935	23	15	10	8	21	1	7	13	31	0	5	26
1935-1940	42	14	13	15	23	0	6	17	27	0	0	27
1941-1946	26	1	8	17	21	0	0	27	18	0	0	18
Total	211	92	60	59	135	21	39	75	140	2	23	115

free of those organisms, and if they did become overgrown it was only by small isolated colonies. We did not see a single gravestone or monument erected in the last 10-12 years with lichens on its surface, while in the same period many basalt gravestones had become more or less covered with them. We saw some individual basalt gravestones which had been grown over with lichens in the last 6 or 7 years. Gravestones made of identical rock have differing degrees of overgrowth. Of 143 basalt gravestones erected from 1884 to 1935, seventy-eight were solidly overgrown and 27 spottily so. The same thing was noted also on gravestones of red and black tufa, but with the difference pointed out above. Here the same phenomenon is observed that we have seen on rocks -- the lichens on the gravestones grow in spots and areas selectively.

It is impossible to explain such a nonuniform growth of lichens on the surface of rocks as a random happening and their selective relationship to individual stones and even blocks. Lichen selectivity, which is noted everywhere in nature under natural conditions and on various manmade structures, is caused by the specific nature of the individual sectors and foci of the rock. These symbiotic organisms grow in the areas of massifs, in the blocks of rock, and on the individual stones which are most favorable to their growth and development. This lichen selectivity may be explained only by the difference in the physicochemical properties of the individual sectors of the rocks, their nutritive value, on the one hand, or the presence of growth-depressing substances, on the other.

The possibility is not excluded that in some cases -- as, for example, on tufa rocks -- the weak lichen development is caused by poisonous antibacterial compounds of certain elements. The indicated differences in the chemical composition of rocks is apparently so slight that they are not successfully discerned by chemical or physical methods. Biological reagents here, as everywhere, prove to be more sensitive.

In a preceding report (1949) we established the fact that on their surface

rocks have a disturbed and altered layer inhabited by a large number of microorganisms. These organisms are the first settlers in the original rocks -- they prepare the soil for other creatures.

In order more precisely to determine the significance of microorganisms in rock destruction and likewise to approach a clarification of the reasons for the focal nature of the distribution of lichens and free-living microorganisms, we conducted special investigations.

In order to ascertain the degree to which rock elements are used as sources of mineral nutriment for microorganisms dwelling in the altered surface layer of the rock, we selected specimens of basalt and red tufa taken from a quarry near the Botanical Garden of the Academy of Sciences of the Armenian SSR. Pieces which were then comminuted into fine powder were taken from a central microbe-free part of the monolithic specimens after the latter had been crushed.

A 5-gram portion of this powder was introduced into 1000 ml distilled water to which were added 0.2 gram of K_2HPO_4, 0.5 gram of NH_4NO_3, and 20 grams of /494 sucrose. No other sources of mineral nutriment were added to the medium, so that these sources would be found by the microorganisms in the introduced basalt rock. Lebedev's findings (1931) were that the following elements are included in the composition of these rocks:

Elements	In Andesitic Basalt (%)	In Tufa Rock (%)
SiO_2	56,05	61,70
TiO_2	0,93	0,86
Al_2O_3	16,91	16,26
Fe_2O_3	2,80	3,60
FeO	4,31	0,70
MnO	0,07	0,09
MgO	5,21	1,44
CaO	7,77	3,79
Na_2O	2,34	3,32
K_2O	2,51	3,97

The thus prepared media were poured into Erlenmeyer flasks in the amount of 100 ml each, and were sterilized at 110°C for 30 minutes in an autoclave. After cooling, the media were inoculated with microbes and put into a dark place at a temperature of 20°C. Inoculation was performed by scraping from the broken-down surface layer of the same rocks, i.e., scraping from basalts was used to inoculate the media with the basaltic dust, and scraping from tufa -- for the media with powdered tufa. The development of the microorganisms in these media was observed.

At certain time-intervals a numerical accounting was made of the growing bacteria. Two series of experiments were set up with three repetitions of each version.

The general picture of microorganism development was that in the first 5-10 days there was no perceptible development in the flasks, and the media remained transparent. There was neither a film nor a gradual ring. Then a turbidity appeared which gradually increased and reached its peak. By this time, fungi were beginning to develop on the surface and first formed a thin film.

This film began to get thicker and thicker and had a grayish or brown color. The surface of the film was covered with an aerial mycelium. During the formation and growth of the film, the medium frequently took on a light brownish-yellow or a dark brown color. The sediment, the rock powder, also became colored. Completion of microorganism growth in the flasks was accompanied by clarification of the medium.

The entire process described takes different periods from one to three months, depending on the growth conditions of the nutrient medium and the inoculated material.

The numerical accounting of microorganism development in the flasks takes place by direct computation. To do this, a drop (0.05 ml) of medium and culture was applied to a slide, evenly spread over an area of one square centimeter, dried, and stained with methylene blue. Thereafter, the number of cells in the field of vision was counted, and the number found was multiplied by the number of fields contained in the preparation. When the culture medium was too thick and saturated with microorganisms, it was first diluted with water and the preparation on the slide was made from this attenuation. In these cases the microbe cells found only in the suspended state were taken into consideration. The cells which developed in the sediment and in the ring on the walls of the vessel or in the film were disregarded, since consideration of them would have required careful mixing or shaking of the contents of the flask, including the rock powder, particles of which would fall onto the preparation and hinder the bacterial cell count.

Table II gives the results of the counts as averages of repeated experiments and analyses. /495

TABLE II. GROWTH OF MICROORGANISMS IN A SYNTHETIC MEDIUM WITH ROCK AS MINERAL NUTRIENT SOURCE (THOUSANDS OF CELLS PER ML OF MEDIUM)

Rocks	Age of Culture in Days							
	0	5	10	20	30	40	60	80
Basalt	0.01	20,0	420,0	8500	120	64	30	30
Tufa	0,01	1.0	10,0	80	200	120	32	10

Microorganism development, as the table shows, proceeds more rapidly and in considerably greater abundance in the basalt medium than in the tufa powder medium. In other words, basalt rock is more favorable to the growth of these creatures.

In addition to the total count, a qualitative group characterization was also made. For this purpose, the culture medium in the above described experiments was inoculated onto agarized nutritive media -- meat-infusion agar, grape must agar, Capek's synthetic medium, and Ashby's anitrogenous medium. (The grape must agar was used for counting fungi).

It its composition, Capek's medium has various mineral salts, nitrogen in the form of a nitrate as the source of nitrogenous nutriment, and sucrose as the source of carbon nutriment. The Ashby medium also consists of different mineral salts, but without nitrogen, while sucrose, glucose, or mannitol is the

source of carbon nutriment.

One drop of culture was inoculated onto these media in Petri dishes, spread with a glass spatula, and after 5-10 days' incubation counted for cultivated colonies. In the flasks where there was too great microorganism growth, the culture was diluted to a certain concentration before inoculation. Inoculation was made from such a flask into two or three dishes with descending culture dilutions. Table III gives the results of these investigations as the average figures of all repeated experiments and analyses.

The research shows that the bulk of the microorganisms developing in our experimental flasks is comprised of bacteria and mycobacteria (approximately 95-98%). There are substantially fewer actinomycetes. Fungi begin to develop at the end of the experiment when the bacteria and mycobacteria have attained their growth. The fungi form a thick film and excrete coloring matter into the medium. The species composition of the fungi differs; for the most part, they belong to imperfect forms (Fungi imperfecti).

Pseudomonas and Bacterium, principally colorless forms, are found among the bacteria.

The numerical ratio of bacteria to mycobacteria varies in different cases. We did not observe a uniform dependence of the numerical microorganism ratio on the rock composition, but in some specimens of basalt we observed predominant development of mycobacteria, sometimes pigmented, i.e., yellow -- M. flavum and yellow-green or lemon yellow -- M. citreum.

The figures given in Table III indicate that the greatest number of microorganisms belongs to the group developing on the Ashby medium; a slightly lower number is discovered on the Capek medium.

The smallest group is composed of the organisms developing on the protein medium (meat-infusion agar).

It must be noted that the colonies of adult organisms on the Ashby agar are generally very small in size, usually no more than 1 mm in diameter. The /496
colonies are often hardly visible to the naked eye, but many are perceptible only under a strong loupe. The colonies are flat and transparent or semitransparent with a slimy surface.

TABLE III. QUALITATIVE CHARACTERISTICS OF MICROORGANISMS GROWN IN A SYNTHETIC MEDIUM WITH ROCK (THOUSANDS OF CELLS PER ML OF MEDIUM)

Age of Culture in Days	Basalt			Tufa		
	Meat infusion Agar	Capek	Ashby	Meat infusion Agar	Capek	Ashby
Initial	0,1	0,1	0,1	0,1	0,1	0,1
5	40	200	640	0,6	1,2	1,8
20	200	6500	12 000	28	180	300
60	64	300	1 000	30	65	100

In subinoculations onto fresh nutrient media, only a few colonies take root. The great majority of them are not successfully transplanted into test tubes and

cultured on any of the numerous experimental media -- simple synthetic (Capek, Bonner, Ashby) and complex organic (meat-infusion, gelatin, bean decoction, must, etc.) media.

Microbiological analysis indicated that the organisms growing on Ashby agar in such small colonies belong principally to three groups -- Bacterium, Pseudomonas, and Mycobacterium.

In the nature of their growth, these organisms resemble those which develop in the surface layer of basalt, granite, and other rocks in situ (Krasil'nikov, 1949).

Thick, slimy, transparent or semitransparent colonies rarely germinate on anitrogenous Ashby agar. They are usually limited in number -- 5-10 per Petri dish or from 100 to 1000 cells per ml of medium. The organisms which form such thick colonies more often belong to sporogenous bacteria and more rarely to the mycobacteria and asporogenous bacteria. In individual cases, their external appearance is reminiscent of colonies of azotobacter. These organisms constitute a special group of oligonitrophylls. They have been little studied and are indubitably of considerable interest.

The bacteria and mycobacteria growing on Ashby agar together with the oligonitrophylls are apparently the most specialized forms. They are adapted to particular conditions of existence on starvation substrata inaccessible to other organisms, where there are neither complex organic materials nor mineral nitrogen in suitable quantity. They are the first settlers of rocks in situ. Alone or in company with free-living algae, they prepare the soil for the following organisms of the plant and animal world.

The group of organisms developing on synthetic Capek medium is also rather numerous (Table III). In species composition, they belong chiefly to Bacterium, Pseudomonas, and Mycobacterium. The principal place belongs now to the first, now to the second, depending on the specimen of rock, its site, and other factors.

This group of organisms also apparently is of great significance in the conquest of primary rocks. Their comparatively small need for sources of organic nutriment gives them the opportunity to develop on starvation and semistarvation media and to have an advantage over the other microorganisms.

The third group of organisms which grows on protein media is not very numerous. It consists principally of asporogenous bacteria -- Pseudomonas, Bacterium; considerably fewer mycobacteria are discovered in this group.

Sporogenous bacteria generally grow very weakly in the media with rocks. In inoculations onto protein media (meat-infusion agar) they comprise no more /497
than 5% of the whole bacterial flora, and more often 0.5-1%.

The third group of microorganisms, as is evident from Table III, is not dominant in the rock microflora. The organisms of this group apparently develop as companions to the first two groups and take advantage of their metabolic products. Their numerical development is conditioned by the presence of the

first two groups, and cannot of course be abundant.

In comparing the species composition of the microflora grown in the basaltic rock medium and the microflora developed in the tufa powder medium, we could not fail to note an essential difference. In both cases three microorganism groups are discovered. We were unable to conduct a more careful differential analysis of this microflora. It is possible that we could not have detected more detailed information on its composition in our analyses. This supposition aroused us to carry out specific research on the nutritive nature of certain monolithic rocks, both well overgrown with lichens and devoid of them.

Basalt specimens collected in the Pushkin Pass in Armenia in 1947 (at a height of about 2000 meters above sea level) were used in the experiments. The rocky summit at the pass is characterized by the fact that some of its blocks of rock are densely covered with a thick layer of different lichens, while some are completely bare, although all are identical in composition. The monoliths of these blocks were taken from the center of the latter, crushed, and ground into a fine powder, which was added to the medium as a source of nutriment (composition of the medium was the same as in the preceding experiments).

After sterilization, the media were inoculated with microflora of the altered surface layer of basalt (scraping from the surface).

The results of these repeated experiments are given in Table IV.* They make clear that the areas of the rock overgrown with lichens are also more favorable to the development of microorganisms than are the areas not covered with lichens.

Conclusions

1. The overgrowth of rocks by lichens and other microorganisms is a focal process.

2. Basalt and tufa rocks were studied as sources of mineral nutriment for microorganisms.

3. It was established that microorganisms grow better on basaltic rocks than on a medium with tufa.

4. Bacteria and mycobacteria undergo the greatest development in media with rocks; actinomycetes and fungi are considerably more feeble in their growth.

All these microorganisms are divided into three groups by the nature of their growth;

(a) Organisms which may be detected by inoculation onto an anitrogenous Ashby medium; together with oligonitrophylls, they constitute the most extensive group.

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*Translators Note: This table not given in the foreign text.

(b) Organisms which may be detected by inoculation onto synthetic media which have mineral nitrogen; they also make up an extensive group of rock microorganisms; and

(c) The least numerous group of bacteria and mycobacteria revealed by inoculation onto protein media (meat-infusion agar).

5. One and the same rock which has been excavated is not homogeneous in chemical composition. There are areas which are overgrown with lichens and which are not. The sections of basalt monoliths, ground into dust and added to the basic medium, exert different effects on the development of rock microorganisms.

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