A SUBTERRANEAN ALGAL FLORA

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That there exists a subterranean algal flora, independent of the terrestrial flora, is a possibility which has seemed so remote that little, if any, attempt has been made to investigate this subject. Many of the earlier writers upon the algae, including Ehrenberg (Mikrogeologie), referred to the algae of the soil, and Gregory,[1] in 1856, discussed somewhat in detail the diatoms obtained from the soil adhering to the roots of dried plants in herbaria.

Robbins,[2] in an account of the algae in some Colorado soils, lists about a dozen blue-greens, one diatom, and two unicellular grass-greens obtained from cultures inoculated with soil. In this case, however, as in all previous accounts, there is no indication that the various forms were not immediately derived from the surface or within a very short distance of the surface of the soil. Robbins removed any loose debris on the surface but the sample consisted of not more than the first three or four inches of earth and included any forms which might have originated terrestrially. These samples, after being thoroughly mixed, were shaken up with distilled water and an amount corresponding to 10 gms. of soil drawn off and distributed over the surface of sterile quartz sand in flasks. Adequate precautions against contamination were observed throughout.

More recently Miss Bristol[3] has reported upon the vitality of algae from old stored soils, but from her account it is obvious

[1] Gregory, W. On the presence of Diatomaceae, Phylolitharia, and sponge spicules in soils which support vegetation. Am. Jour. Sci. and Arts II. **21**: 434–437. 1856.

[2] Robbins, W. W. Algae in some Colorado soils. Colo. Agr. Exp. Sta., Bull. **184**: 24–36. *pl. 1–4.* 1912.

[3] Bristol, B. M. On the retention of vitality by algae from old stored soils. New Phytol. **18**: 92. 1919.

that only samples from the surface were used, and with a single exception any reference in the literature to soil algae may be regarded as having only to do with those forms which grow at or near the surface. Esmarch,[1] however, in a rather extensive paper, attempted to indicate not only the distribution of *Cyanophyceae* upon the surface of various soils but also their occurrence underground. His method was to use Petri dishes about 2 cm. deep, into which 1 cm. of the soil to be studied was introduced. The soil was moistened with sterile water and a piece of filter-paper placed over the surface. The cultures were kept in the greenhouse with diffuse light at a temperature of from 20 to 25° C., and after periods of from two days to two months growth appeared through the filter-paper.

In investigating the distribution of blue-green algae on surface soils an attempt was made to determine whether cultivation influenced their distribution. Accordingly, 4 types of uncultivated soils, including sandy meadow, marshy bog, forest humus, and moist sand, were investigated. On the sandy meadow, which contained traces of humus, but 3 out of 34 samples collected showed the presence of *Cyanophyceae* on the surface. On the marshy bog soil, after a period of three months, none of the 35 cultures showed any blue-green algae, although a few diatoms and grass-green algae were present. Both the forest humus and the moist sand gave good results, so far as indicating the presence of numerous blue-greens on the surface. In cultivated soils, 3 types were used, namely, sandy, clay, and marshy. Of these, 29 out of 45 samples of sandy soil contained *Cyanophyceae*, comprising some 12 different kinds. On the clay soil, 35 out of 37 samples produced blue-green algae with 23 species. On the marshy soil, of the 40 cultures all but 2 showed growth, 22 species being found. While, in general, the above increase indicates that a greater number of blue-greens were found on cultivated than uncultivated soils, the number of samples was so few and taken from such a limited area that no very exact conclusions could be drawn. Any difference in

[1] Esmarch, F. Untersuchungen über die Verbreitung der Cyanophyceen auf und in verscheidenen Böden. Hedwigia **55**: 224–273. 1914.

the two types of soil seemed to be determined by the moisture content and the mineral nutrient content.

Coming to the question of the presence of algae underground, Esmarch continued to attempt to correlate their growth with different types of soil. Samples were taken at about the same place as those used for surface cultures, at a depth usually of from 10 to 25 cm. A few, however, extended from 30 to 50 cm. below the surface. All samples were obtained in a manner to prevent surface contamination. The results are grouped according to the kind of soil. In tilled land 13 cultures were made from sandy soil, 12 from clay, and 20 from marshy soil. Only 5 of these contained no blue-green algae and were from places where there were no surface forms. In all, 18 separate species were found and the number decreased as we went deeper into the soil. In the meadow land 23 out of 32 cultures contained blue-greens, with 15 species represented, all these occurring on the surface as well as underground. In moist sand practically all cultures gave results, with 20 different species showing growth. The brown heath and bog soils produced no blue-greens from below the surface.

Esmarch records the occurrence of these subterranean forms as due to the distribution of surface organisms by seepage of surface waters or by being carried down by earth worms and other soil organisms, and, although he was inclined to believe that the blue-greens found by him beneath the surface could grow in the absence of light, he does not regard the work of other investigators on this subject as being altogether conclusive. Furthermore, Esmarch doubts that the blue-green filaments found at considerable depths in the soil have been able to persist there for any length of time. In order to demonstrate this, he prepared cultures in Petri dishes containing 7–8 mm. of soil, on which a piece of filter-paper was placed with certain blue-greens on the surface. The filter-paper was then covered with about 1 cm. of soil, the cultures moistened with distilled water and covered with black paper, the whole being placed in a light-proof case. The temperature was maintained at from 15 to 20° C. After a longer or shorter time, depending upon the

character and mineral content of the soil as well as upon individual differences of the algae themselves, the filaments became discolored, passing from a pale blue-green through a yellowish green to yellow. At first the contents of the cells appeared normal and were apparently in a healthy condition. Later the filaments disintegrated, leaving only spores and heterocysts behind. Cultures which showed practically no normal filaments were removed from the light-proof case, the moistened filter-paper placed on top of the soil, and after about 12 weeks' exposure to daylight again showed blue-green growth. Esmarch regarded this experiment as definitely indicating the impossibility of blue-greens persisting beneath the surface for any length of time, and considered that while the absence of light was a factor, the destructive influence of the soil itself must be taken into consideration.

Aside from this paper of Esmarch's, there appears to be no record of algae growing at considerable depths in the soil, and the investigation here recorded—a preliminary announcement of which was made at the Pittsburgh meeting of the Botanical Society of America, on December 29, 1917—is believed to be the first definite indication that there may exist in the soil, at depths up to 1 m., at least one grass-green alga which is practically always present as a subterranean organism under conditions which preclude its having recently been derived from the surface and accidentally carried down to various depths.

METHODS

The method employed throughout this study was essentially the following:

About 1½ inches of sand was placed in pint milk bottles, to which was added 150 cc. of a culture solution. The bottles were plugged with cotton and sterilized at 8–10 pounds pressure for ½ hour. The culture solution was prepared ½ the strength of the formula of a modified Beyerinck's solution used by Moore,[1] because of the soluble material present in the sand.

[1] Moore, G. T. Methods for growing pure cultures of algae. Jour. Appl. Microsc. 6: 2309–2314. 1903.

The formula undiluted was:

Ammonium nitrate	.5 gm.
Monobasic potassium phosphate	.2 gm.
Magnesium sulphate	.2 gm.
Calcium chloride	.1 gm.
Iron sulphate	Trace
Water	1000 cc.

The bottles were inoculated in duplicate under sterile conditions with about 10 gms. of soil taken from various depths. Every precaution was taken so that the exposed surface was not contaminated with small particles of soil carried down from the upper layers. The spatula by which the samples were taken was sterilized after each inoculation. Checks were run with bottles that were exposed to the air where the inoculations were taken. In order to lessen the amount of evaporation waxed paper covers were placed over the cotton plugs. The sand was slanted in the bottle so that part of it was not submerged, in this way giving various moisture conditions in the culture. The cultures were then placed in cases where they received good light for at least part of the day. The water lost by evaporation was restored from time to time with sterile water.

In order to compare the algal flora of different regions and soil conditions, 10 different series of bottles were inoculated with soil samples from various parts of the Missouri Botanical Garden, 1 from Woods Hole, Massachusetts, and 3 from the vicinity of Santa Ana, California. The varieties of soil examined were heavy clay, loose clay, sand, sandy alkali, sandy gravel and humus. All subterranean cultures were obtained from places where the soil had not been disturbed for at least a number of years. This precaution was necessary in order that the algal growths obtained would represent those typical of subterranean conditions and not merely recent surface infections. At no time did a single check culture show growth, thus eliminating the possibility of algal infection from the air.

SERIES B

INOCULATED OCTOBER 1, 1915. SAMPLE FROM MISSOURI BOTANICAL GARDEN, NORTHWEST CORNER OF LINNEAN HOUSE WALL. FILLED IN TO 45 CM.; BLACK HUMUS TO 20 CM.; BELOW THIS A GRADUAL CHANGE TO PURE CLAY; VERY FINE CLAY AT 37 CM.; DEPTH LIMIT 100 CM.

Ser. B	Depth	Feb. 24, 1916	Mar. 29, 1916	Apr. 11, 1916	June 7, 1916	Aug. 25, 1916	Nov. 14, 1916
B′	Surface			*Protoderma viride*	*P. viride* (motile) *Stichococcus bacillaris*	*P. viride* (motile) *S. bacillaris*	*P. viride* (motile) *S. bacillaris*
B′	Surface		*P. viride*	*P. viride*	*P. viride* (motile)	*P. viride* (motile) *S. bacillaris* *Ulothrix variabilis*	*P. viride* Diatoms
B″	Surface				*P. viride*	*P. viride* (motile) *S. bacillaris* *U. variabilis*	*P. viride*
1	10 cm.		*P. viride* (motile)	*P. viride* *Cladophora* sp. Diatoms	*P. viride* *Cladophora* sp. Diatoms	*P. viride* *Trochiscia?* *U. variabilis* Diatoms	*P. viride* *U. variabilis* Diatoms
1′	10 cm.			*P. viride*	*P. viride*	*P. viride*	*P. viride* Diatoms
2	20 cm.		*P. viride* (motile) Diatoms	*P. viride* (motile) Diatoms	*P. viride* (motile) Diatoms	*P. viride* (motile) Diatoms	*P. viride* (motile) Diatoms
2′	20 cm.						*P. viride* (motile) Diatoms

SERIES B—*Continued*

Ser. B	Depth	Feb. 24, 1916	Mar. 29, 1916	Apr. 11, 1916	June 7, 1916	Aug. 25, 1916	Nov. 14, 1916
3	30 cm.			*P. viride*	*P. viride* (motile)	*P. viride*	*P. viride* *S. bacillaris*
3′	30 cm.						*P. viride* *S. bacillaris*
4	40 cm.	*P. viride*	*P. viride*	*P. viride* (motile)	*P. viride*	*P. viride*	*P. viride* (motile) Diatoms
4′	40 cm.						
5	50 cm.		*P. viride* *Cladophora* sp.	*P. viride* *Cladophora* sp.	*P. viride* *Cladophora* sp.	*P. viride* *Cladophora* sp.	*P. viride*
5′	50 cm.		*P. viride*	*P. viride*	*P. viride*	*P. viride* Diatoms	*P. viride* Diatoms
6	60 cm.						Diatoms
6′	60 cm.						Diatoms
7	70 cm.						
7′	70 cm.					*P. viride* Diatoms	*P. viride* Diatoms
7″	70 cm.						*S. bacillaris* Diatoms

8 (80 cm.), 9 (90 cm.), and 10 (100 cm.), no growth.

SERIES C

INOCULATED OCTOBER 1, 1916. SAMPLE FROM MISSOURI BOTANICAL GARDEN, HOLE IN CENTER OF LINNEAN HOUSE. SURFACE STONY AND MOIST; FILLED IN TO 20 CM. WITH CLAY, LIME AND BRICK; 20–40 CM., A MIXTURE OF CLAY AND HUMUS; 40–100 CM., GRADUAL CHANGE FROM CLAY TO VERY FINE CLAY. CULTURES C3 AND C3′ WERE TAKEN IN A TAR-LIKE STRATA; DEPTH LIMIT 100 CM.

Ser. C	Depth	Jan. 14, '16	Mar. 25, '16	Aug. 22, '16	Nov. 10, '18
C	Surface		*Ulothrix variabilis* *Stichococcus bacillaris*	*Protoderma viride* *S. bacillaris* *U. variabilis* *Trochiscia?*	*P. viride* *S. bacillaris* *Trochiscia?* *U. variabilis* (plasmolyzed)
C′	Surface	*P. viride* *U. variabilis* *S. bacillaris*	*P. viride* *U. variabilis* *S. bacillaris*	*P. viride* *S. bacillaris* *U. variabilis* *Trochiscia?*	*P. viride*
C″	Surface		*P. viride*	*P. viride* *S. bacillaris* *U. variabilis* *Trochiscia?*	*P. viride* *U. variabilis*
1	10 cm.			*P. viride*	*P. viride*
1′	10 cm.			*P. viride*	*P. viride*
2	20 cm.			*P. viride*	*P. viride*
2′	20 cm.			*P. viride*	*P. viride*
3	30 cm.			*P. viride*	*P. viride*
3′	30 cm.				*P. viride* *U. variabilis*
4	40 cm.		*P. viride*	*P. viride*	*P. viride*
4′	40 cm.				*P. viride*
5	50 cm.			*P. viride*	*P. viride*
5′	50 cm.	*P. viride*	*P. viride*	*P. viride*	*P. viride* *S. bacillaris*
6	60 cm.		*P. viride*	*P. viride*	*P. viride*

SERIES C—*Continued*

Ser. C	Depth	Jan. 14, '16	Mar. 25, '16	Aug. 22, '16	Nov. 10, '18
6′	60 cm.			*P. viride*	*P. viride*
7	70 cm.		*P. viride*	*P. viride*	*P. viride*
7′	70 cm.				*P. viride*
8	80 cm.				*P. viride* Diatoms
8′	80 cm.				*P. viride* (scant growth)
9	90 cm.		*P. viride*	*P. viride* (motile)	*P. viride*
9′	90 cm.				
10	100 cm.				
10′	100 cm.			*P. viride*	*P. viride* (motile)

SERIES D

INOCULATED MAY 10, 1916. SAMPLE FROM MISSOURI BOTANICAL GARDEN, CUT IN NEW EMBANKMENT ALONG ROADSIDE EAST OF NEW PROPAGATING HOUSES. NATURAL FORMATION; BLACK HUMUS TO 20 CM.; CLAY TO VERY FINE CLAY THE REMAINING DEPTH; DEPTH LIMIT 120 CM.

Ser. D	Depth	June 10, 1916	June 17, 1916	Aug. 24, 1916	Mar. 8, 1917	Nov. 22, 1918
D	Surface	*Protoderma viride*	*P. viride* *Cladophora* sp. *Ulothrix variabilis*	*P. viride* *Cladophora* sp. *U. variabilis* Diatoms	*P. viride* *U. variabilis* *Stichococcus bacillaris* Diatoms	*P. viride* *Cladophora* sp. *U. variabilis* *S. bacillaris* Diatoms
D	Surface			*P. viride*	*P. viride*	*P. viride*
1	10 cm.		*P. viride* *Cladophora* sp.	*P. viride*	*P. viride* *U. variabilis*	*P. viride*
1′	10 cm.		*P. viride* *Cladophora* sp.	*P. viride* *Cladophora* sp.	*P. viride*	*P. viride* (plasmolyzed)
2	20 cm.	*P. viride*	*P. viride* *Cladophora* sp.	*P. viride* *U. variabilis*	*P. viride* *U. variabilis*	
2′	20 cm.		*P. viride* *Cladophora* sp.	*P. viride*	*P. viride*	*P. viride*
3	30 cm.	*P. viride*	*P. viride* *Cladophora* sp.	*P. viride* *Cladophora* sp.	*P. viride*	*P. viride*
3′	30 cm.	*P. viride*	*P. viride* *Cladophora* sp.	*P. viride* *Cladophora* sp.	*P. viride*	
4	40 cm.		*P. viride*	*P. viride* *U. variabilis*	*P. viride* *U. variabilis*	
4′	40 cm.	*P. viride*	*P. viride*	*P. viride*	*P. viride* Diatoms	

SERIES D—*Continued*

Ser. D	Depth	June 10, 1916	June 17, 1916	Aug. 24, 1916	Mar. 8, 1917	Nov. 22, 1918
5	50 cm.			*P. viride*	*P. viride*	*P. viride* Diatoms
5′	50 cm.		*P. viride*	*P. viride* Diatoms	*P. viride* Diatoms	
6	60 cm.					
6′	60 cm.			*P. viride*	*P. viride*	*P. viride*
7	70 cm.					
7′	70 cm.			*P. viride*	*P. viride*	*P. viride*
8	80 cm.					
8′	80 cm.			*P. viride* Diatoms	*P. viride* Diatoms	*P. viride* Diatoms
9	90 cm.			*P. viride* Diatoms	*P. viride* Diatoms	*P. viride* Diatoms

100 (100 cm.), 110 (110 cm.), and 120 (120 cm.), no growth.

SERIES E

INOCULATED JUNE 6, 1916. SAMPLE FROM MISSOURI BOTANICAL GARDEN, HOLE IN NORTH AMERICAN TRACT. NATURAL FORMATION; BLACK HUMUS TO 20–30 CM.; BELOW THIS CLAY TO VERY FINE CLAY; DEPTH LIMIT 80 CM.

Ser. E	Depth	Sept. 15, '16	Dec. 16, '16	Mar. 29, '17	Nov. 16, '18
E	Surface		*Protoderma viride*	*P. viride* *Ulothrix variabilis*	*P. viride* *U. variabilis*
E′	Surface			*P. viride* *U. variabilis*	Diatoms
E″	Surface			*P. viride* *U. variabilis* *Cladophora* sp.	*P. viride*
1	10 cm.		*P. viride*	*P. viride*	*P. viride*
1′	10 cm.			*P. viride*	*P. viride*
2	20 cm.		*P. viride*	*P. viride* *U. variabilis*	*P. viride*
2′	20 cm.			*P. viride* *U. variabilis*	
3	30 cm.		*P. viride*	*P. viride*	*P. viride*
3′	30 cm.			*P. viride*	*P. viride*
4	40 cm.			*P. viride*	*P. viride*
4′	40 cm.			*P. viride*	*P. viride*
5	50 cm.			*P. viride*	*P. viride*
5′	50 cm.				
6	60 cm.		*P. viride*	*P. viride*	*P. viride*
6′	60 cm.				
7	70 cm.			*P. viride*	*P. viride*
7′	70 cm.				*P. viride*
8	80 cm.				

SERIES F

INOCULATED JUNE 6, 1916. SAMPLE FROM MISSOURI BOTANICAL GARDEN, HOLE, AT EDGE OF WOODED NORTH AMERICAN TRACT, DUG UNDER TREES. NATURAL FORMATION; BLACK HUMUS TO 20–30 CM.; REMAINING DEPTH CLAY TO VERY FINE CLAY; DEPTH LIMIT 70 CM.

Ser. F	Depth	Aug. 25, 1916	Sept. 15, 1916	Sept. 19, 1916	Mar. 30, 1917	Nov. 19, 1916
F	Surface		*Protoderma viride*	*P. viride* *Ulothrix variabilis*	*P. viride*	*P. viride*
F′	Surface		*P. viride*	*P. viride* *U. variabilis* *Stichococcus bacillaris*	*P. viride* (motile)	*P. viride*
F″	Surface		*P. viride*	*P. viride* *U. variabilis*	*P. viride* (motile)	*P. viride* (motile)
1	10 cm.		*P. viride* *U. variabilis*	*P. viride*	*P. viride* *U. variabilis*	*P. viride*
1′	10 cm.		*P. viride*	*P. viride*	*P. viride*	*P. viride*
2	20 cm.		*P. viride* *U. variabilis*	*P. viride* *U. variabilis*	*P. viride* *U. variabilis*	*P. viride* *U. variabilis*
2′	20 cm.		*P. viride* *U. variabilis*	*P. viride* *U. variabilis*	*P. viride* *U. variabilis*	*P. viride* *U. variabilis*
3	30 cm.		*P. viride*	*P. viride*	*P. viride*	*P. viride*
3′	30 cm.		*P. viride* *Trochiscia?*	*P. viride* Diatoms	*P. viride*	*P. viride*
4	40 cm.		*P. viride*	*P. viride* Diatoms	*P. viride* Diatoms	*P. viride* Diatoms
4′	40 cm.			*P. viride*	*P. viride*	*P. viride*
5	50 cm.		*P. viride*	*P. viride* *U. variabilis*	*P. viride*	*P. viride* *U. variabilis*
5′	50 cm.			*P. viride*		*P. viride* *U. variabilis*

SERIES F—*Continued*

Ser. F	Depth	Aug. 25, 1916	Sept. 15, 1916	Sept. 19, 1916	Mar. 30, 1917	Nov. 19, 1916
6	60 cm.		*P. viride* *Trochiscia?*	*P. viride* *U. variabilis*		*P. viride* *U. variabilis*
6′	60 cm.			*P. viride* *U. variabilis*		*P. viride*
7	70 cm.		*P. viride*	*P. viride*		
7′	70 cm.					

SERIES G

INOCULATED SEPTEMBER 26, 1916. SAMPLE TAKEN FROM MISSOURI BOTANICAL GARDEN, NEWLY EXCAVATED TRENCH PARALLEL WITH LINNEAN HOUSE WALL. PACKED ROAD-BED INTERMIXED WITH TAR TO 10 CM.; NATURAL FORMATION AT 20 CM.; REMAINING DEPTH GRADUALLY GRADING INTO CLAY; DEPTH LIMIT 40 CM.

Ser. G	Depth	Nov. 2, '16	Mar. 12, '17
G	Surface	*Protoderma viride* *Stichococcus bacillaris* *Ulothrix variabilis*	*P. viride* *S. bacillaris* *U. variabilis*
G′	Surface	*P. viride* *Cladophora* sp. *S. bacillaris* *U. variabilis*	*P. viride* *Cladophora* sp. *S. bacillaris* *U. variabilis*
1	10 cm.	*P. viride* *Cladophora* sp.	*P. viride* *Cladophora* sp.
1′	10 cm.	*P. viride* *Cladophora* sp.	*P. viride* *Cladophora* sp.
2	20 cm.	*P. viride*	*P. viride*
2′	20 cm.	*P. viride*	*P. viride*
3	30 cm.	*P. viride*	*P. viride*
3′	30 cm.	*P. viride*	*P. viride*
4	40 cm.	*P. viride*	*P. viride*
4′	40 cm.	*P. viride*	*P. viride*

SERIES H

INOCULATED OCTOBER 12, 1918. SAMPLE FROM MISSOURI BOTANICAL GARDEN, NEWLY EXCAVATED AREA IN NORTH END NEAR SERVICE SHOPS. CLAY AND HUMUS TO 25 CM.; REMAINING DEPTH CLAY; DEPTH LIMIT 100 CM.

Ser. H	Depth	Nov. 20, '18	Jan. 21, '19	Mar. 21, '19	July 10, '19
H	Surface		*Protoderma viride* *Ulothrix variabilis*	*P. viride* *Nostoc muscorum* *Oscillatoria formosa* *O. anoema* *O. splendida* *Scytonema Hofmanni* *Navicula atemoides* *Nitzschia Kützingiana* *Hantzschia amphioxys*	*P. viride* *N. muscorum* *O. formosa* *O. anoema* *O. splendida* *S. Hofmanni* *N. atemoides* *N. Kützingiana* *H. amphioxys* *Stichococcus bacillaris*
H′	Surface				
1	5 cm.	*P. viride*	*P. viride* *N. muscorum* *O. chlorina* *N. atemoides* *N. Kützingiana*	*P. viride* *N. muscorum* *O. chlorina* *N. atemoides* *N. Kützingiana* *Cladophora* sp.	*P. viride* *N. muscorum* *O. chlorina* *N. atemoides* *N. Kützingiana* *Cladophora* sp.
1′	5 cm.		*P. viride* *N. muscorum* *S. Hofmanni* *O. amphibia* *O. subtilissima* *N. atemoides* *N. Kützingiana*	*P. viride* *N. muscorum* *S. Hofmanni* *O. amphibia* *O. subtilissima* *N. atemoides* *N. Kützingiana*	*P. viride* *N. muscorum* *S. Hofmanni* *O. amphibia* *O. subtilissima* *N. atemoides* *N. Kützingiana*
2	10 cm.	*P. viride*	*P. viride* *N. muscorum* *O. amphibia* *O. subtilissima* *N. atemoides* *N. Kützingiana*	*P. viride* *N. muscorum* *O. amphibia* *O. subtilissima* *N. atemoides* *N. Kützingiana*	*P. viride* *N. muscorum* *O. amphibia* *O. subtilissima* *N. atemoides* *N. Kützingiana*
2′	10 cm.	*P. viride*	*P. viride* *N. muscorum* *O. amphibia* *O. subtilissima* *N. atemoides* *N. Kützingiana* *Cladophora* sp.	*P. viride* *N. muscorum* *O. amphibia* *O. subtilissima* *N. atemoides* *N. Kützingiana* *Cladophora* sp.	*P. viride* *N. muscorum* *O. amphibia* *O. subtilissima* *N. atemoides* *N. Kützingiana* *Cladophora* sp.

SERIES H—*Continued*

Ser. H	Depth	Nov. 20, '18	Jan. 21, '19	Mar. 21, '19	July 10, '19
3	15 cm.	*P. viride*	*P. viride* *O. amphibia* *O. subtilissima* *N. atemoides* *N. Kützingiana* *Hantzschia amphioxys*	*P. viride* *O. amphibia* *O. subtilissima* *N. atemoides* *N. Kützingiana* *H. amphioxys*	*P. viride* *O. amphibia* *O. subtilissima* *N. atemoides* *N. Kützingiana* *H. amphioxys*
3′	15 cm.	*P. viride* *O. amphibia* *O. subtilissima* *S. Hofmanni* *N. atemoides* *N. Kützingiana* *H. amphioxys*	*P. viride* *O. amphibia* *O. subtilissima* *S. Hofmanni* *N. atemoides* *N. Kützingiana* *H. amphioxys*	*P. viride* *O. amphibia* *O. subtilissima* *N. atemoides* *S. Hofmanni* *N. Kützingiana* *H. amphioxys*	*P. viride* *O. amphibia* *O. subtilissima* *N. atemoides* *S. Hofmanni* *N. Kützingiana* *H. amphioxys*
4	20 cm.	*P. viride*	*P. viride* *U. variabilis*	*P. viride* *U. variabilis* *O. amphibia* *O. chlorina* *O. subtilissima*	*P. viride* *U. variabilis* *O. amphibia* *O. chlorina* *O. subtilissima*
4′	20 cm.	*P. viride*	*P. viride* *U. variabilis* *O. amphibia* *O. chlorina* *O. subtilissima* *N. muscorum* *S. Hofmanni* *N. atemoides* *N. Kützingiana*	*P. viride* *U. variabilis* *O. amphibia* *O. chlorina* *O. subtilissima* *N. muscorum* *S. Hofmanni* *N. atemoides* *N. Kützingiana*	*P. viride* *U. variabilis* *O. amphibia* *O. chlorina* *O. subtilissima* *N. muscorum* *S. Hofmanni* *N. atemoides* *N. Kützingiana*
5	25 cm.		*P. viride*	*P. viride*	*P. viride*
5′	25 cm.				
6	30 cm.		*P. viride* *U. variabilis* *Cladophora* sp. *N. atemoides*	*P. viride* *U. variabilis* *Cladophora* sp. *N. atemoides*	*P. viride* *U. variabilis* *Cladophora* sp. *N. atemoides*
6′	30 cm.	*P. viride*	*P. viride* *N. atemoides*	*P. viride* *N. atemoides*	*P. viride* *N. atemoides*
7	35 cm.	*P. viride*	*P. viride* *N. atemoides*	*P. viride* *U. variabilis* *N. atemoides*	*P. viride* *U. variabilis* *N. atemoides*
7′	35 cm.	*P. viride*	*P. viride* *N. atemoides*	*P. viride* *N. atemoides*	*P. viride* *N. atemoides*

SERIES H—*Continued*

Ser. H	Depth	Nov. 20, '18	Jan. 21, '19	Mar. 21, '19	July 10, '19
8	40 cm.	*P. viride*	*P. viride*	*P. viride*	*P. viride*
8′	40 cm.	*P. viride*	*P. viride*	*P. viride*	*P. viride*
9	45 cm.	*P. viride*	*P. viride*	*P. viride*	*P. viride*
9′	45 cm.	*P. viride*	*P. viride*	*P. viride* *Cladophora* sp.	*P. viride* *Cladophora* sp.
10	50 cm.	*P. viride*	*P. viride*	*P. viride*	*P. viride*
10′	50 cm.	*P. viride*	*P. viride* *Trochiscia*?	*P. viride*	*P. viride*
11	55 cm.	*P. viride*	*P. viride* *Trochiscia*?	*P. viride*	*P. viride*
11′	55 cm.	*P. viride*	*P. viride*	*P. viride*	*P. viride*
12	60 cm.		*P. viride* *Cladophora* sp.	*P. viride* *Cladophora* sp.	*P. viride* *Cladophora* sp.
12′	60 cm.	*P. viride*	*P. viride*	*P. viride*	*P. viride*
13	65 cm.	*P. viride*	*P. viride*	*P. viride*	*P. viride*
13′	65 cm.		*P. viride*	*P. viride*	*P. viride*
14	70 cm.				
14′	70 cm.				
15	80 cm.				
15′	80 cm.		*P. viride*	*P. viride*	*P. viride*
16	90 cm.		*P. viride*	*P. viride*	*P. viride*
16′	90 cm.		*P. viride*	*P. viride*	*P. viride*
17	100 cm.		*P. viride*	*P. viride*	*P. viride*
17′	100 cm.		*P. viride*	*P. viride*	*P. viride*

SERIES J

INOCULATED JUNE 27, 1919. SAMPLE FROM MISSOURI BOTANICAL GARDEN, ABOUT 10 CM. FROM THE PLACE IN SERIES H; DEPTH LIMIT 100 CM.

Ser. J	Depth	Sept. 23, '19	Nov. 4, '19
J	Surface	*Protoderma viride* *Nostoc muscorum* *Hantzschia amphioxys*	*P. viride* *N. muscorum* *H. amphioxys*
J′	Surface	*P. viride*	*P. viride*
1	5 cm.	*P. viride*	*P. viride* *Cladophora* sp. *H. amphioxys*
1′	5 cm.	*P. viride*	*P. viride* *Cladophora* sp. *H. amphioxys*
2	10 cm.	*P. viride* *Ulothrix variabilis*	*P. viride* *U. variabilis* *Cladophora* sp.
2′	10 cm.	*P. viride* (motile)	*P. viride* *Navicula atemoides*
3	15 cm.	*P. viride*	*P. viride* *N. atemoides*
3′	15 cm.	*P. viride*	*P. viride* *N. atemoides*
4	20 cm.		
4′	20 cm.		
5	25 cm.	*P. viride*	*P. viride* *N. atemoides*
5′	25 cm.	*P. viride*	*P. viride*
6	30 cm.	*P. viride* (motile)	*P. viride*
6′	30 cm.	*P. viride*	*P. viride*
7	35 cm.		*P. viride*

SERIES J—*Continued*

Ser. J	Depth	Sept. 23, '19	Nov. 4, '19
7′	35 cm.	*P. viride*	*P. viride*
8	40 cm.	*P. viride*	*P. viride*
8′	40 cm.	*P. viride*	*P. viride*
9	45 cm.		
9′	45 cm.		
10	50 cm.	*P. viride*	*P. viride*
10′	50 cm.	*P. viride*	*P. viride*
11	55 cm.	*P. viride* *N. atemoides*	*P. viride* *N. atemoides*
11′	55 cm.	*P. viride* *N. atemoides*	*P. viride* *N. atemoides*
12	60 cm.	*P. viride*	*P. viride* *Oscillatoria amphibia*
12′	60 cm.	*P. viride*	*P. viride*
13	65 cm.	*P. viride*	*P. viride*
13′	65 cm.	*P. viride* (motile)	*P. viride* *Trochiscia?*
14	70 cm.	*P. viride* *H. amphioxys* *N. atemoides*	*P. viride* *H. amphioxys* *N. atemoides* *O. amphibia*
14′	70 cm.	*P. viride* *H. amphioxys* *N. atemoides*	*P. viride* *H. amphioxys* *N. atemoides*
15	75 cm.		*P. viride*
15′	75 cm.		

SERIES J—*Continued*

Ser. J	Depth	Sept. 23, '19	Nov. 4, '19
16	80 cm.	*P. viride* (motile) *Trochiscia?*	*P. viride* (motile) *Trochiscia?*
16′	80 cm.	*P. viride*	*P. viride* (motile)
17	85 cm.	*P. viride*	*P. viride*
17′	85 cm.	*P. viride* *N. atemoides*	*P. viride* *N. atemoides*
18	90 cm.	*P. viride*	*P. viride*
18′	90 cm.	*P. viride*	*P. viride* *H. amphioxys* *N. atemoides*
19	95 cm.	*P. viride* (motile)	*P. viride* *N. atemoides*
19′	95 cm.	*P. viride*	*P. viride* *N. atemoides*
20	100 cm.		*P. viride* *H. amphioxys* *N. atemoides*
20′	100 cm.		*P. viride* *H. amphioxys* *N. atemoides*

Series A, inoculated with very poor soil from the Missouri Botanical Garden, was a preliminary series and not carefully examined, so that no record is tabulated. Cultures were taken at the surface and at 20, 40, and 60 cm. below the surface. *Protoderma viride* was found in all the cultures and *Anabaena* appeared in those taken at a depth of 20 cm.

Series H contained a greater number of blue-green forms than any of the other series, and it seemed desirable to repeat the experiment. Series I was inoculated March 6, and Series J, June 27, 1919, with soil taken within a few centimeters of the area used in Series H. The surface of the embankment was scraped off to expose a clean area. At the end of 3 months in Series I, and 40 days in Series J, no growth was apparent, whereas in Series H growth had been abundant in almost all of the bottles in the latter period of time.

Since Series I showed no growth at the end of 90 days, the cultures were discarded. Growth in Series J first appeared at the end of about 40 days. Even at the end of 3 months these cultures showed less growth and fewer species of algae than those of Series H. However, *Protoderma viride* again appeared throughout. The fact that there was no growth in Series I and that fewer species of algae appeared in Series J may have been due to the surface of the embankment having been exposed during the winter months and the low temperature probably having killed some of the forms originally present in Series H. This exposure probably killed many or all of the vegetative cells of the algae which survived, and thus the delayed growth in Series J might be explained by the persistence of spores which required a longer period of time in which to produce a visible growth.

The cultures of soil from Woods Hole, Mass. and Santa Ana, Calif. were taken in exactly the same manner to a depth of 1 meter as the preceding ones. The soil at Woods Hole was sandy gravel containing several large boulders. The series taken at Santa Ana were especially valuable because of the different soil conditions, one series being taken from very sandy soil and another from sandy alkali soil. The third series was taken

from ordinary garden soil. No tabulated results were kept of these because *Protoderma* appeared in all of the cultures.

From the above tables, it will be seen that there exists a subterranean algal flora independent of the nature of the soil and the locality. A wide variety of algae does not appear in the soils examined but in most cases the variety is as great as at the surface. The absence of a variety of blue-green algae and the constant occurrence of *Protoderma viride* is especially noticeable. The fact that the latter occurs at the greatest depth and in every soil seems to indicate that it is especially adapted to live under subterranean conditions.

The greater number of soil samples studied in this investigation is comparable to those termed uncultivated forest soils by Esmarch. The results in general are also similar in that he found no *Cyanophyceae* on the surface or underground. The soils in all cases were uncultivated, a fact which may account for fewer cultures showing blue-green forms than reported by Robbins and Esmarch. It is possible that the unicellular green alga reported by Robbins is a form of *Protoderma viride*.

As has been pointed out by Esmarch, this flora undoubtedly originated from the surface flora, but its persistence in the soil at such great depths is noteworthy. It is inconceivable that in undisturbed soil compact as clay, algae could be carried down very far by surface waters. There were no evidences of wormholes or penetration by surface organisms in these soils. This would seem to indicate that the algae are in a vegetative condition and actually grow in the soil.

The amount of growth in the various bottles can be taken to represent in a general way the abundance of the algae in the soil at the different depths, since the cultures were all kept under similar conditions. It was impossible to determine this from a microscopical examination of the soil samples, because the algae were present in such small quantities that they could not be easily found among the soil particles. The greatest growth was never at the surface but at a depth of 5–60 cm. This was due probably to the dry conditions existing at the surface. From 60 to 100 cm. the amount of algae in the cultures gradu-

ally became less. In some cases this was due to the disappearance of some of the algal forms but usually the amount of an individual form also decreased. *Protoderma* was always more abundant towards the surface than at the greater depths.

The time in which the growth was first perceptible in the cultures varied from about 3 weeks to 3 months. This was dependent no doubt upon the amount of algae and also upon the amount in a vegetative condition in the soil. Obviously, vegetative cells would produce a growth in less time than spores.

The resistance of these algae to desiccation was demonstrated in series B–F inclusive, in which the cultures were allowed to evaporate for a period of about 18 months, from March, 1917, to November, 1918. The cultures became quite dry within several months, so that the algae were exposed to desiccating conditions for about 12 months. In the fall of 1918, the cultures were reëxamined and in most cases the algae seemed in a healthy condition. After the cultures were moistened vigorous growth occurred again. Especially noticeable was the fact that while many of the vegetative cells of *Ulothrix* and *Stichococcus* were plasmolyzed, very few of the *Protoderma* cells showed any injurious effects.

The following is a list of the algae found in the cultures and the greatest depth at which they occurred:

Protoderma viride Kützing	100 cm.
Hantzschia amphioxys (Ehr.) Grun	100 cm.
Navicula atemoides Grun	100 cm.
Trochiscia?	80 cm.
Stichococcus bacillaris Nägeli	70 cm.
Oscillatoria amphibia Agardh	70 cm.
Cladophora sp	60 cm.
Ulothrix variabilis Kützing	60 cm.
Anabaena sp	20 cm.
Nitzschia Kützingiana Hilse	20 cm.
Nostoc muscorum Agardh	20 cm.
Oscillatoria chlorina Kützing	20 cm.
Oscillatoria subtilissima Kützing	20 cm.
Scytonema Hofmanni Agardh	20 cm.
Oscillatoria anoema (Kützing) Gomont	Surface
Oscillatoria formosa Bory	Surface
Oscillatoria splendida Greville	Surface

Artificial Subterranean Cultures

Very little work has been done on the effect of subterranean conditions upon the growth of algae. In studying the effect of light, culture media have been used which did not duplicate soil conditions. Esmarch determined the effect of light upon the growth of certain *Cyanophyceae* which he found in subterranean cultures. As shown above, these algae were grown on soil in the dark and examined from time to time. From his results, he concluded that these forms could live in darkness for a short period of time but after several weeks, in most cases, the cells showed effects due to the absence of light. The extent of this effect depended upon the nature of the soil and individual characteristics of the alga. Eventually only spores and heterocysts remained.

Since *Protoderma viride* was found to be universally present in the cultures, an attempt was made to determine the effect of subterranean conditions upon its growth. The foregoing results showed that it could exist in undisturbed soils for a depth of one meter at least, thus being capable of living for long periods of time in the absence of light. In order to determine the effect of these conditions upon its growth, the following experiments were performed:

One culture was set up January 15, 1919. A small amount of sterile clay was put into a sterile glass cylinder about 2 cm. in diameter and 1 m. long. The soil was moistened with sterile water and a piece of sterile filter-paper that had been inoculated with *Protoderma* derived from culture H9′ was placed on top. Alternate layers of soil, moistened with water, and inoculated filter-paper were added until the tube was filled. A sterile cotton plug was used to cork the top, so that some aëration could take place. The tube was then sunk in the ground to the depth of 1 m., thus allowing the culture to grow under somewhat natural conditions. A flower-pot was placed over the top to protect the cotton plug.

Other cultures were set up on February 1, 1919. The actual aëration conditions occurring in the soil were more nearly duplicated in these than in the previous culture, since small sterile

cheese-cloth bags were filled with sterile clay soil which had been inoculated with *Protoderma* from culture H8′. These were placed in 6 sterile atmometer tubes which were then filled with sterile soil and enough sterile water to moisten. The tubes were corked and put in the ground at a depth of about 25 cm.

The tube in the first experiment was examined after about five months, on June 20, 1919, and *Protoderma* was found upon the filter-paper and also to some extent in the soil. Abundant growth was obtained in the part of the tube at the surface where some light had entered because the flower-pot did not at all times fit closely over the top. The rate of growth beneath the surface was much less, but even at a depth of 1 m. the algal cells were brilliantly green and healthy and had grown to some extent into the soil. This is comparable to the growth occurring under natural conditions, because in the soil samples taken algal growth was so scant that it could not be detected with the naked eye. In all cases the cells were either in plates or small and large single cells, some of which resembled *Protococcus*.

In the second experiment, the tubes were examined after different intervals of time. This method proved to be less satisfactory than the above due to the difficulty of finding the scattered algal cells among the soil particles. Thus, negative results would not necessarily mean that the algal cells had disintegrated.

Culture No.	Time examined	Interval	Result
1	Feb. 12, 1919	11 days	Some brown and colorless cells.
2	Feb. 18, 1919	17 days	No cells.
3	Feb. 24, 1919	23 days	Numerous green cells.
4	June 20, 1919	140 days	No cells.
5 and 6	June 27, 1919	147 days	Numerous green cells (2 tubes examined).

The above results agree with those in the first experiment. The fact that numerous *Protoderma* cells were found in the cul-

ture kept underground for the longest period of time would seem to indicate that the first culture examined was not a normal one. In cultures 2 and 4, the cells may have been overlooked, owing to the difficulty of finding them among the soil particles.

Since there is now in progress a detailed physiological study of *Protoderma* which will attempt to determine its possible function in the soil together with the influence of various environmental factors on its life history and growth, no reference to the literature nor further discussion of the problem need be given here. It is hoped that a subsequent paper on the subject may be published in the Annals within a short time.