

The Agricultural Experiment Station  
OF THE  
Colorado Agricultural College

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I. THE AMMONIFYING EFFICIENCY OF CERTAIN  
COLORADO SOILS

BY

WALTER G. SACKETT

II. ALGAE IN SOME COLORADO SOILS

BY

W. W. ROBBINS

# The Agricultural Experiment Station

FORT COLLINS, COLORADO

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# THE AMMONIFYING EFFICIENCY OF CERTAIN COLORADO SOILS.

BY WALTER G. SACKETT.

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## INTRODUCTION.

In a former bulletin (1) the writer has called attention to the power of many cultivated Colorado soils to fix atmospheric nitrogen through the agency of *Azotobacter*, both in the soil and in mannite solutions. The investigation referred to was undertaken for the purpose of determining the source of the nitrogen from which the excessive nitrates, present in some of our soils, might have been derived. The results of this work point clearly to the atmosphere as the source of the nitrogen and to *Azotobacter* as the medium by which it is transformed from a gaseous into a proteid form, and subsequently transferred as such to the soil.

With an ample and reasonably constant supply of protein thus assured, our efforts have been directed, more recently, toward a study of the transformation of the combined nitrogen into ammonia the first step in the formation of nitrates from the complex nitrogen molecule. In the present investigation, we have determined the ammonifying efficiency of some thirty Colorado soils, many of which are known to be affected with the niter trouble. I use the term *ammonifying efficiency* in the sense in which it has been employed by Stevens (2), to denote not only the presence of ammonifying organisms in the soil which are capable of exercising their specific function under favorable conditions, but also the suitability of the soil as a medium in which the process of ammonification may proceed advantageously.

The soils under examination have been collected from a wide range of territory representing orchard land, sugar beet, oat and alfalfa fields, barren wastes and raw land. They include a variety of soil types, and almost all have been under cultivation and irrigation at one time or another.

The proteid nitrogen for our experiments has been supplied in four different forms:—Cottonseed meal, dried blood, alfalfa meal and flaxseed meal. These have been employed rather than soluble forms such as peptone and gelatin since the latter could not be used in a practical way under field conditions, and, furthermore, the results obtained from substances of this kind would be of little practical value outside of the pure scientific interest attached. On the other hand, by making use of some of the more common nitrogenous fertilizers, we have been able to learn something of the availability of the nitrogen in these materials

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(1) Bacteriological Studies of the Fixation of Nitrogen in Certain Colorado Soils. Bul. 179 Colorado Experiment Station, 1911.

(2) Stevens, F. L. and Withers, W. A., Studies in Soil Bacteriology III. Concerning methods for determination of nitrifying and ammonifying powers. Cent. f. Bakt. Abt. II., Bd. 25, No. 1-4, p. 64, 1909.

with reference to our soils, and consequently we are in a better position to recommend their use when such a practice becomes necessary.

### METHODS.

In collecting the soil samples, every precaution has been taken to eliminate exterior contaminations. All surface debris was removed before opening up the soil, and all instruments and containers were thoroughly sterilized. Unless otherwise stated, the samples were taken to a depth of three inches; the soil in each case was removed with a sterilized spatula and placed in double, sterilized, paper sugar sacks. All samples were shipped by express to the bacteriological laboratory of the Experiment Station in order to minimize the time in transit, during which interval, if unduly prolonged, the soil flora might undergo changes. This statement is deemed necessary since many of the samples were taken more than five hundred miles from Fort Collins. Immediately upon arrival at the laboratory, each soil was spread out upon a sheet of heavy, sterilized, manilla paper and thoroughly mixed. It was next divided into two unequal portions, the larger part being allowed to dry in the air in diffused light, while the remaining portion was transferred in a moist condition to a sterilized Mason fruit jar. As soon as the soils were air dry, which seldom requires more than twenty-four hours in our atmosphere, each was ground in a glass mortar, sterilized with mercuric chloride and subsequently rinsed with boiled, distilled water, and passed through a thirty mesh wire sieve. From each sample prepared in this manner, ten 100-gram portions were weighed out, and eight of these were transferred to deep culture dishes, 10 x 4 cm., similar to the ordinary Petri dish only deeper; the two remaining lots were analyzed at once for ammonia. A weighed amount of each of the four nitrogenous materials, employed to furnish the organic nitrogen, corresponding to 100 m. g. of total nitrogen was added to each of two 100 gram portions of soil. It was thoroughly mixed with the soil by constant stirring with a sterilized glass rod for five minutes. Each preparation was then inoculated with 10 c. c. of its respective soil infusion, corresponding to 5 grams of the fresh soil. The infusions were made by shaking 100 grams of undried soil with 200 c. c. of sterile, distilled water, and after allowing it to stand for thirty minutes for the coarser particles to settle, the required amount of the turbid suspension was drawn off with a sterile pipette and distributed uniformly over the surface of the soil in the culture dish. In addition, each basin received sufficient sterile, distilled water to give the soil its optimum moisture content, approximately 20 per cent. Additional quantities of water were used for the organic matter at the rate of 1.5 c. c. for each gram of material. All of the cultures were kept in the incubator for seven days at a temperature of 28° - 30° C. At the end of this time, the contents of each dish were transferred to a copper distilling flask with 250 c. c. of ammonia—free water and distilled with 7 grams of heavy, calcined magnesia to liberate the free ammonia. The distillates were received in N/10 sulphuric acid and subsequently titrated with

standard solutions to determine the amount of ammonia formed during the experiment.

The various nitrogenous substances employed to furnish the proteid nitrogen contained total nitrogen as follows:

Cottonseed meal.....	7.8463 per cent. total nitrogen
Dried blood.....	13.0503 per cent. total nitrogen
Alfalfa meal.....	2.5053 per cent. total nitrogen
Flaxseed meal.....	3.7507 per cent. total nitrogen

To obtain 100 m. g. of total nitrogen from these materials the following amounts were taken:

Cottonseed meal.....	1.2744 grams
Dried blood.....	0.7662 grams
Alfalfa meal.....	3.9915 grams
Flaxseed meal.....	2.6661 grams

The ammonia originally present in the soils was determined, and, although of negligible quantity in many cases, corrections have been made for it in the results of the analyses. The ammonia found is given in Table No. 3, page 19.

## HISTORY, CHARACTER AND AMMONIFYING EFFICIENCY OF THE SOILS UNDER STUDY.

### SAMPLE No. 1.

The orchard from which this sample was obtained was first brought to my attention in the summer of 1910 because of the appearance of niter burning on some of the apple trees. This is an old orchard, and two of the trees were in a very serious condition at that time. I visited it again in the fall of 1911, when I collected the present sample, and both of the trees affected in 1910 were dead, while seven others, all Ben Davis, were in a critical state. The soil is a heavy, adobe clay and was moist from recent rains. The nitrogen fixation test in mannite solution, made one year previously, gave an increase of 11.3483 m. g. of nitrogen per 100 c. c. of solution in thirty days. The nitrogen recovered as ammonia from the different organic materials in seven days was as follows:

From cottonseed meal 46.63%; dried blood 37.02%; alfalfa meal 17.55%; flaxseed meal 3.01%.

### SAMPLE No. 2.

This represents a portion of another orchard in a heavy clay soil adjacent to No. 1. No losses had been incurred here as yet from niter, although at this time, fall 1911, six large trees were unquestionably affected. A young orchard to the north, with alfalfa between the rows of trees, was in a very thrifty condition. An adjoining orchard of possibly ten to twelve acres belonging to the same owner had suffered considerable injury from niter for the past three years. The land had been manured heavily, but, so far as

checking the destruction, no benefit could be observed.

The ammonification test with this soil showed the following amounts of nitrogen recovered as ammonia in seven days:

From cottonseed meal 42.31%; dried blood 47.04%; alfalfa meal 12.78%; flaxseed meal 8.09%.

#### SAMPLE No. 3.

Sample No. 3 is a sandy loam, and was obtained from a large orchard where niter burning was first observed in 1910. The number of trees involved was rather large, but the damage done up to the spring of 1912 had not been serious. This is one of the few tracts where the trouble is present, and yet where it has made little real progress. Each succeeding year a few more trees become affected, but the orchard, as a whole, is holding its own. Oats had been sown as a shade crop when I sampled the soil in the fall of 1911. The ammonification tests gave the following percentages of nitrogen recovered in seven days as ammonia:

From cottonseed meal 25.92%; dried blood 18.03%; alfalfa meal 12.06%; flaxseed meal 6.30%. In view of the small amount of injury and the slow rate at which it is moving, the relatively low ammonifying efficiency as brought out by these results is very interesting. Compared with the two preceding samples taken from orchard were dead, and the trees on the remaining acre were in ammonification of cottonseed meal and dried blood was less than half as rapid. If the same holds true of nitrification, it is easy to understand why the nitrates have not become excessive as yet.

#### SAMPLE No. 4.

This soil comes from an orchard where no excessive niter had manifested itself previous to 1911. In driving through the country, I had passed by this place frequently in former years, but had never observed anything unusual about either the trees or the soil. The high nitrates had been very destructive within half a mile of this locality, and whole orchard tracts, embracing ten to twenty acres, had been wiped out. By October, 1911, two acres of this orchard were dead, and the trees on the remaining acre were in all stages of burning. The soil, a sandy loam, was very brown both in the orchard and along the ditch banks. I have not seen it yet this year, but I should be very much surprised to find a single tree alive. The results of the ammonification tests on this soil point again to the close relation between excessive nitrates, as measured by the destruction of vegetation, and the high ammonifying efficiency. The following percentages of nitrogen were recovered as ammonia from the different nitrogenous materials:

From cottonseed meal 44.62%; dried blood 46.93%; alfalfa meal 12.40%; flaxseed meal 1.12%.

## SAMPLE NO. 5.

Here we have another bearing orchard of probably twenty acres, seven of which had been killed by niter previous to 1911, and the trees from that portion had been pulled out. The land had been planted to oats in the spring of 1911, but judging from the scattering stubble which I saw in the fall, the original stand had been very poor. Many more trees were either dead or dying at this time, and the prospects were that the entire tract would be gone by the end of 1912. My sample, a sandy loam, was taken beside a badly burned tree in that part of the orchard where the injury was most active at that time. The nitrogen recovered as ammonia with this soil was as follows:

From cottonseed meal 38.63%; dried blood 36.78%; alfalfa meal 21.08%; flaxseed meal 20.10%. It will be seen from these figures that the yields from the alfalfa and flaxseed meals are much higher than those obtained with any other soil. Because of an unavoidable delay, the ammonia determinations on this series were not made until after eleven days, and the prolonged incubation period will probably account for the increase obtained here.

## SAMPLE NO. 6.

This soil presents a very interesting history. It comes from a forty acre tract, twenty acres of which had been in alfalfa, and the remainder was bearing orchard. In 1907, barren spots began to appear here and there in the alfalfa, and brown patches on the soil, indicative of niter, were observed in the orchard. It was not long before the trees commenced to die in a manner that we have since come to associate with excessive nitrates. Here, as we have noticed so frequently elsewhere, a few trees in the innermost part of the orchard succumbed first, and with these as a focal center, the trouble spread with such marvelous rapidity that by the spring of 1909 all of the alfalfa had been destroyed and fifty per cent. of the trees were lost. The year 1909 saw the remaining trees perish, save for parts of six rows on one side of the orchard next to a ditch. During 1910, the three inside rows were killed, and the fourth and fifth were burning. During 1911 the fourth and fifth died and the sixth was burning. (Fig. 1.) I am sure I do not know where we could find a more beautiful illustration of the formation and spread of nitrates from a central point than is given by the regular succession in which row after row of trees went down before the approaching wave of niter. From 1909 to 1911, the orchard was a barren waste, where absolutely nothing would grow, not even the commonest weed. (Fig. 2) The *Azotobacter* flora had been exterminated entirely from the surface layers of this area, although soil taken near one of the surviving trees in row five next to the outside row mentioned above gave a vigorous growth of *Azotobacter* and a fixation of 12.4689 m. g. of nitrogen per 100 c. c. of mannite solution.

When I visited this place in the fall of 1911 to secure my sample

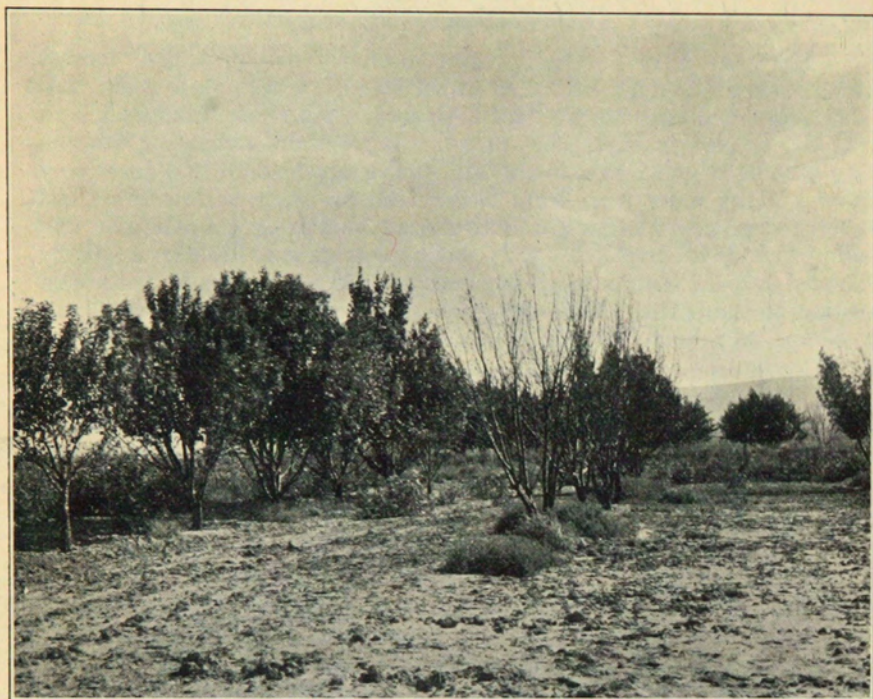


Figure 1.



Figure 2.





Figure 3.

for ammonification, I was overwhelmed with astonishment, to put it mildly, to see the whole barren portion almost entirely covered with saltbush (*Atriplex*) waist high. Unfortunately, before I was able to obtain a photograph of this, the owner had burned over the area to destroy the weeds. However, I did get a picture later, after the fire had swept across, which will convey to the reader some idea of the luxuriance of the vegetation, although it gives no adequate conception of the height of the growth. (Fig. 3.) During the winter and spring of 1910 and 1911, the precipitation was unusually heavy in this region. The accumulation of nitrates in the surface layers had evidently been carried down by leaching until the concentration of the salts had been reduced to a point where the weeds could grow, and when once established, they had utilized the nitrates to the best of their ability in making a rank growth. This was the first instance in which we had ever observed anything that even suggested self reclamation of a niter area. Since then, no other locality has come to our notice.

The soil is a clay loam, and the sample for the ammonification experiment was taken between two burning trees in the last surviving row. The results of the examination give the following per-

centages of nitrogen recovered as ammonia from the nitrogenous fertilizers:

From cottonseed meal 43.47%; dried blood 23.55%; alfalfa meal 8.72%; flaxseed meal .10%.

#### SAMPLE NO. 7.

This sample was obtained from an orchard where the niter trouble has been very severe for the past three years. The first trees died in 1908, and the owner, believing that they were short of plant food, had given that section of the orchard a heavy dressing of stable manure. The next year, the attack started in with renewed vigor, in spite of the fertilizer, and has grown rapidly worse until five or six acres of a once profitable orchard are worthless. The soil is a sandy loam and the ammonification test gave the following results:

From cottonseed meal, 46.40% of the total nitrogen was recovered as ammonia; dried blood 32.75%; alfalfa meal 10.61%; flaxseed meal 3.99%.

#### SAMPLE NO. 8.

This soil was obtained from a young orchard which has been reset for the past eight or nine years with the hope of getting a successful stand. Many of the trees died the same year that they were put out, while some have struggled along for three and four seasons just able to keep alive. Occasionally, a tree is found which shows no symptoms of niter and which is making a good growth. The space between the trees is planted to alfalfa, and in many parts of the orchard barren spots are visible. Before this shade crop was put in, one had no difficulty at all in discerning the brown color of the soil and the dark stains on the irrigating furrows, so characteristic of *A. chroococcum*. The soil is a clay loam and the sample for the ammonification experiment was taken from a bare spot where a tree had died.

The percentages of nitrogen recovered as ammonia were as follows:

From cottonseed meal 51.98%; dried blood 47.98%; alfalfa meal 15.30%; flaxseed meal 1.12%.

#### SAMPLE NO. 9.

I visited this orchard the last time in the fall of 1911 at picking season, and the picture it presented was indeed a deplorable sight. Tree after tree had died loaded with half grown fruit. Many were bending to the ground with beautiful red apples, but there was not enough vitality left in the body to bring them to maturity. Occasionally there was a tree, scattered among these, which appeared perfectly normal, and again there would be those that showed the injury in a mild degree, possibly a little burning on the water sprouts or on a small limb. Five acres of the orchard were lost during 1910 and at least three acres more last year. The dis-

tribution of the trouble was different in this case from what we ordinarily find; the trees were not dying in any particular section as a whole, but were scattered throughout the tract, alternately good and bad. This was the first and most serious outbreak of niter in this region, which is approximately twenty miles from the orchards described previously. My sample was collected near a dying tree on October 4, 1911. The soil is a loam, inclining to clay. The results from the ammonification test are as follows:

From cottonseed meal 36.25% nitrogen recovered as ammonia;  
dried blood 32.46%; alfalfa meal 12.08%; flaxseed meal .87%.

#### SAMPLE NO. 10.

Two years ago, while looking over the orchard just described, I was called into a neighboring orchard to pass an opinion on some dying apricot trees. A glance at the soil revealed the brown stain of niter on the irrigating furrows, and a dozen burning apple trees confirmed the observation. I took a sample of this soil and found that it was capable of fixing 10.15725 m. g. of nitrogen per 100 c. c. of mannite solution in thirty days. Before leaving the orchard, I hunted around rather carefully to see if there were many trees in a serious condition, but so far as I could discover they were all confined to a limited section of two rows. When I went back there last October to get another sample of soil for my ammonification work, I was unable to locate either the brown soil or the affected trees, and a diligent search failed to reveal any more trees which were suffering. The sample which I secured was taken in a peach orchard adjacent to the block of apples referred to, and to the best of my knowledge represents a normal orchard soil, if the vigor of peach trees can be taken as any indication. It might be described as a loam, inclining to clay. The following results were obtained in the ammonification test:

From cottonseed meal 28.33% of the nitrogen was recovered as ammonia; dried blood 23.57%; alfalfa meal 4.97%; flaxseed meal 8.15%.

#### SAMPLE NO. 11.

Sample No. 11 was collected in October, 1911, from an orchard some distance from any that has been described previously, and until this season no niter trouble had been in evidence. About thirty trees in all, in one corner, were dying in a typical fashion. The soil is a clay loam and was rather moist from a recent shower, making it difficult to determine the presence of any brown color. The percentages of nitrogen recovered as ammonia in the ammonification test were as follows:

From cottonseed meal 47.58%; dried blood 51.17%; alfalfa meal 13.59%; flaxseed meal 12.15%.

#### SAMPLE NO. 12.

In the spring of 1910, the trees from about four and one-half

acres of this orchard were pulled up and consigned to the wood pile, and the land was planted to corn. This was another case of a twenty year old orchard killed by niter in less than two years. The corn failed to make any growth, and much of it never came through the ground. The whole surface was covered with a hard, brown crust beneath which the soil was mealy and ashy in character. The soil is an elegant sandy loam, with splendid natural drainage. More as an experiment than anything else, this ground was planted to cantaloupes in 1911. Here and there a plant became established and succeeded fairly well, but the crop as a whole was a failure. This spring, 1912, the tract was planted to oats, notwithstanding the brown, mealy condition of the soil. The grain which is immediately adjacent to the irrigating furrows, where the niter appears to have been partially removed, seems to be making a pretty good growth, but that between the furrows, where the niter is still concentrated, is at a standstill. The ammonification results on this sample give the following percentages of nitrogen recovered as ammonia:

From cottonseed meal 38.81%; dried blood 20.67%; alfalfa meal 7.19%; flaxseed meal .38%.

#### SAMPLE NO. 13.

This soil is a clay loam from an alfalfa field and was selected from a locality where the nitrate trouble has been serious in neighboring orchards. Material collected from this same piece of ground in 1910 fixed 10.15925 m. g. of nitrogen in thirty days per 100 c. c. of mannite solution, so there is no question about the presence of *Azotobacter*. A chemical analysis of the soil does not show excessive nitrates. The alfalfa is perfectly healthy, is making a splendid growth, and, so far as the eye can detect, both the crop and the soil are normal. The sample for ammonification was secured March 27, 1912. The results of the test show the following percentages of nitrogen recovered as ammonia:

From cottonseed meal 45.11%; dried blood 41.15%; alfalfa meal 7.36%; flaxseed meal 7.71%.

#### SAMPLE NO. 14.

The next soil comes from an orchard on the edge of a mesa one hundred and fifty feet above the surrounding country. Ten apple trees had died here in 1910 with all the symptoms of niter and about fifty more in 1911. The soil is a clay loam in good condition of tilth with no evidence of any brown color due to *Azotobacter* or other signs indicative of excessive nitrates save the burning of the apple leaves. To all appearances, the trouble is in the incipient stage. The ammonification test follows: From cottonseed meal 47.73% nitrogen was recovered as ammonia; dried blood 52.33%; alfalfa meal 16.56%; flaxseed meal 3.99%.

#### SAMPLE NO 15.

About one mile back on the mesa mentioned above, is an area where the high nitrates have done a great deal of damage the past two

years, particularly to bearing apple orchards. This sample comes from such a place, where it was thought at first that the injury was due to faulty drainage. Accordingly, in 1910, an experienced engineer was employed to put in the proper amount of tile at the correct depth, but the trees have continued to die in spite of the drain. Practically two of the seven acres of this orchard are worthless today. Many of the trees were dead outright when I saw the place last fall, while others were struggling along with just life enough to put out a dwarfed, stunted foliage. The soil is a sandy loam and was collected near a burning tree, October 24, 1911. The ammonification tests give the following percentages of nitrogen recovered as ammonia in seven days: From cottonseed meal 49.07%; dried blood 50.78%; alfalfa meal 15.86%; flaxseed meal 1.82%.

#### SAMPLE NO. 16.

This sample was obtained from an orchard in the same region as Number 15, but not adjoining it. The soil is a red, sandy loam, and because of this peculiar color it has always been rather difficult to detect any brown discoloration, although there is no question about the excessive nitrates for nearly twenty acres of bearing orchard have been ruined since 1910. Here, as in the preceding orchard, the trees appear to be dying gradually rather than going in one season as is the case so often. Near the farm house where the surface of the soil has not been disturbed by cultivation, the characteristic brown color and mealy condition are quite apparent. The nitrogen fixing power of this soil in 1910 amounted to 7.1451 m. g. of nitrogen per 100 c. c. manite solution in thirty days. The ammonification experiment gave the following amounts of nitrogen recovered as ammonia in seven days: From cottonseed meal 47.10%; dried blood 52.64%; alfalfa meal 13.69%; flaxseed meal .21%.

#### SAMPLE NO. 17.

After giving considerable attention to the biological activities in cultivated soils, I was interested in knowing whether raw adobe clay, which had never received any cultivation, and which had never been disturbed since the time it was formed by the weathering of the underlying shale possessed any ammonifying powers. A previous examination for *Azotobacter* had failed to show the presence of this genus. To this end, I selected an adobe hill where this type of topography prevailed, in a section of the country where agriculture was absolutely out of the question. The hill was about eight miles from the nearest town, a half mile from the wagon road, inaccessible, and arose abruptly from the edge of a stream to a height of 150 to 200 feet. Because of its location, I doubt if many human beings had ever ascended it, and, in fact, I see no reason for anyone to have done so unless on a mission similar to mine. There was no vegetation whatever upon it, and aside from a few bird tracks and one lonely spider, I saw no evidence of animal visitations. While collecting my sample from the highest point of the hill, I noticed numerous pockets of white crystals, pre-

sumably calcium sulphate, distributed through the soil. Although the very surface was dry, the clay was moist below the top half inch. I was indeed surprised to learn from the results of the ammonification work on this soil that the cottonseed meal had given up 37.37% of its nitrogen as ammonia; dried blood 23.67%; alfalfa meal 12.75%; flaxseed meal 2.87%.

#### SAMPLE No. 18.

This sample comes from a field which had been in sugar beets in 1910 and in oats in 1911. The soil is a hard clay with considerable gravel, and the crops have not done well in late years because of the poor drainage conditions. The underlying shales appear to have formed a series of basins which retain the irrigating waters and thus interfere with natural drainage. Recently, extensive tile drains have been laid, and the trouble from excessive water should soon be lessened. In addition to the seeped condition of the land, niter has done some damage on this mesa, although not in the field which we are considering now. My purpose in taking a sample of this soil was to have something to compare with the next sample which was obtained from a neighboring field where both water and niter had been destructive. The ammonification results follow:

From cottonseed meal, 28.02% of the nitrogen was recovered as ammonia; dried blood 39.79%; alfalfa meal 2.83%; flaxseed meal 5.26%.

#### SAMPLE No. 19.

This represents a field which was planted to barley in 1910 but the nitrates which had been accumulating for years had become so concentrated by this time that nothing could grow except next to the irrigating furrows where the water appears to have reduced the salts to a degree of partial tolerance. In 1910, the soil, a gravelly clay, was dark brown and mealy beneath the surface crust. When I visited the ranch in March, 1912, extensive drains were being installed, but it was too early to expect any benefit. The moist condition of the soil made it rather difficult at this time to detect the characteristic brown color, so prominent in the years before. However, the soil was becoming mealy in spots as it dried out. The ammonification results obtained from this sample are as follows:

From cottonseed meal 44.72% of nitrogen was recovered as ammonia; dried blood 47.74%; alfalfa meal 13.06%; flaxseed meal 2.55%. A comparison of these figures with those of the preceding sample is quite striking when it is remembered that No. 18 is the same kind of a soil secured from a nearby field, but where the niter had not manifested itself. Soil No. 18 liberated only 30.68% of the nitrogen of cottonseed meal as ammonia, while No. 19 set free 45.70%; the former gave 42.45% with dried blood the latter 46.72%; the former 5.49% with alfalfa meal; the latter 14.04%; the better soil gave higher returns from the flaxseed meal, the ratio being 7.92 to 3.53.

## SAMPLE NO. 20.

Our next case presents a very interesting history. In 1908 the field was planted to oats but it was only a short time until a number of brown, mealy patches, on which nothing would grow, developed on the higher places. It should be mentioned in passing that seepage had given some trouble in former years, and for that reason the growing of alfalfa on that piece of ground had been abandoned. In 1909 the land was planted to sugar beets, but the stand was very poor; there were great barren areas of half an acre in extent where nothing would grow. These bare spots were decidedly brown and mealy. The beet crop was almost a total failure, and that fall the field was seeded to winter wheat. The spring of 1910 brought no relief, for the whole twenty-five acres of wheat perished long before harvest. The greater part of the tract remained a barren waste all that summer, with not even a Russian thistle growing on it. As has been mentioned before, the precipitation for the winter and spring of 1910 and 1911 was unusually heavy and prolonged, and whether it was due to the leaching resulting from this, or to some other unknown cause, I know not, but in 1911 the whole area blossomed out in a most luxuriant growth of saltbush and Russian thistles chest high. So far as our present observations go, this field and No. 6 are the only instances where niter areas have shown any tendency toward recovery. The surface of the soil was moist and green with a moss protonema when I took my sample in October, 1911. It is a clay loam and mealy in spots beneath the brown crust. The results of the ammonification experiment are as follows:

From cottonseed meal, 48.23% nitrogen was recovered as ammonia in seven days; dried blood 38.98%; alfalfa meal 9.81%; flaxseed meal 5.07%.

## SAMPLE NO. 21.

Sample No. 21 was taken in the fall of 1911 from an orchard where the niter injury was first observed in 1909. During 1909 and 1910 approximately two and a half acres had been killed, and the remainder of the trees were unquestionably affected in 1911 but the progress of the trouble seemed to have been retarded from some cause. In place of the trees being entirely destroyed in a month to six weeks, as is frequently true, these dragged along, half leaved out and sickly looking, throughout the season. I am unable to say whether they came out in leaf this spring or not. I obtained a sample of this soil in 1910 and found it to possess marked nitrogen fixing powers. In thirty days it gave an increase of 9.807 m. g. of nitrogen per 100 c. c. of mannite solution. The soil is a clay loam and shows the brown stain of the *Azotobacter* pigment readily. The ammonification test made from soil secured in October, 1911, gave the following:

From cottonseed meal 47.87% nitrogen recovered as ammonia; dried blood 49.16%; alfalfa meal 12.22%; flaxseed meal .77%.

## SAMPLE NO. 22.

This sample comes from a ninety acre orchard, where the trees have been dying from excessive nitrates since 1908. During the winter of 1911 and 1912, the manager took out approximately two hundred and fifty dead apple trees from one corner of the orchard to say nothing of those removed here and there from other parts. A nitrogen fixation test made on this soil two years ago gave an increase of 8.89635 m. g. of nitrogen per 100 c. c. mannite solution in thirty days. At this time, the characteristic brown stain was very perceptible on the irrigating furrows, and today in some parts of the orchard the entire surface bears this same color. The orchard had been seeded to oats as a shade crop when I took my first sample for ammonification on October 27, 1911. The grain was about knee high and the stand was very thin. It was raining hard at this time, so it was impossible to tell anything about either the brown color or physical condition of the soil. The soil is a sandy loam and was collected beside a burning tree in that section of the orchard which was subsequently grubbed out. The results of the ammonification experiment are as follows:

From cottonseed meal 39.89% nitrogen was recovered as ammonia; dried blood 31.38%; alfalfa meal 11.63%; flaxseed meal 1.54%.

## SAMPLES NOS. 23, 24, 25 AND 26, 27, 28.

The samples were all collected from the orchard described as No. 22 and represent two vertical sections. Two large trenches had been dug to ascertain the level of the ground water; one in the lowest part of the section from which the trees had been removed, and the other back in the orchard on higher ground where the trees were just beginning to burn. In the first hole the water plane was found to be four feet eight inches from the surface, while in the second, no water was struck at eight feet. Judging by the eye, the latter was in ground four to five feet higher than the former. The face of each trench was cut down as smooth and clear as possible with a shovel, and then the surface inch of this face was removed at the point where the sample was to be taken with a sterile spatula. After cutting out this surface block very carefully, the sample proper was taken with a second sterilized spatula. These precautions were taken in order to avoid the danger from surface contaminations which were almost certain to have been carried down with the shovel. In this manner, three samples were obtained from each hole at three different depths; namely, (1) the surface three inches; (2) 18th to 24th inches; (3) 56th to 60th inches. Samples Nos. 23, 24, and 25 came from hole No. 1, and Nos. 26, 27, and 28 from No. 2. Nos. 23 and 26 represent the surface portions; Nos. 24 and 27, the section at 18 to 24 inches; and Nos. 25 and 28, the samples at 56 to 60 inches from the respective holes.

The soils are so unlike in physical character at the different



depths in the two excavations that a brief description is necessary to a clear understanding and correct interpretation of the results obtained in the ammonification experiments. No. 23 is a sandy loam, more or less mealy from excessive niter; No. 24 is a mixture of sand and gravel with abundant moisture; No. 25 was taken near the bottom of the hole in the water bearing stratum, and consists of coarse sand and gravel, thoroughly saturated with water. The top 13 inches of soil from hole No. 2 was frozen and sample No. 20, taken from this portion, is a sandy loam, not mealy; No. 27 is a clean, sharp, dry gravel with neither sand nor soil present. This material is so coarse that practically nothing passed through a wire sieve with 20 meshes to the inch, and in preparing the sample, it was put through a 10 mesh sieve instead of the usual 30 mesh; No. 28 is a fine, moist sand with almost no gravel.

The ammonification results with these six soils are given in the following table.

*Table No. 1. Ammonification by Samples Nos. 23 to 28.*

No.	Source	Per cent. nitrogen recovered		as ammonia in 7 days from:	
		Cottonseed meal	Dried blood	Alfalfa meal	Flaxseed meal
23	Surface 3 in.	45.32	30.47	17.93	6.20
24	18 to 24 in.	48.29	39.25	26.27	1.12
25	56 to 60 in.	47.45	34.04	15.41	.84
26	Surface 3 in.	50.85	44.76	26.24	9.53
27	18 to 24 in.	22.62	46.37	34.85	.91
28	56 to 60 in.	38.53	46.72	28.82	.87

Comparing the results from the surface samples of the two holes, Nos. 23 and 26, it is very clear that the excessive nitrates in the former have depressed ammonification. On the whole, ammonification has been more active in the soils outside of the heavy niter area. The ammonification of flaxseed meal is accomplished almost entirely by the surface flora, this function disappearing very rapidly in the first two feet. Bacteria capable of ammonifying cottonseed meal, dried blood and alfalfa meal appear to occur almost uniformly throughout the first five feet. No. 24 gave the highest yields of ammonia of any of the samples from hole No. 1, except with flaxseed meal, due, possibly, to its loose, open texture. No. 27 gave the highest percentages of ammonia from dried blood and alfalfa meal, but was strikingly deficient in the microorganisms necessary for the destruction of cottonseed meal. No. 26 gave the largest amounts of ammonia from cottonseed meal and flaxseed meal. The large percentages of ammonia produced by Nos. 25 and 28 are worth noting in view of the fact that these are both deep soils in which one would hardly expect to find active ammonifying bacteria.

#### SAMPLE No. 29.

A truck garden on the outskirts of a mining town furnished the next sample. This soil is of particular interest since previous to its present ownership, it was held as a placer gold claim. The elevation is some 3000 feet higher than the country from which the

Table No. 2. *The Ammonifying Efficiency of Certain Colorado Soils.*

No.	Source	Character of Soil	Total nitrogen used—100 mgs.		Per cent, nitrogen recovered as ammonia in 7 days from:		Per cent, nitrogen recovered as ammonia in 7 days from:	
			Cottonseed meal		Dried blood		Flaxseed meal	
			Per cent.	mgs.	Per cent.	mgs.	Per cent.	mgs.
1	Orchard	Heavy ashobe clay	46.86	46.33	37.06	36.99	37.02	17.55
2	Orchard	Heavy ashobe clay	43.85	40.77	42.31	47.00	47.04	17.55
3	Orchard	Sandy loam	25.50	26.34	25.92	18.06	18.00	12.54
4	Orchard	Sandy loam	15.11	44.13	44.62	46.79	47.07	12.06
5	Orchard	Sandy loam	38.81	38.46	38.63	36.85	36.71	12.06
6	Orchard	Clay loam	43.36	43.59	43.47	23.12	23.89	12.40
7	Orchard	Sandy loam	46.37	46.44	46.40	33.48	32.62	21.08*
8	Orchard	Clay loam	51.98	51.98	51.98	48.54	47.42	20.59
9	Orchard	Clay loam	36.78	35.73	36.25	31.87	33.06	8.72
10	Orchard	Clay loam	28.30	28.37	28.33	23.47	23.68	8.69
11	Orchard	Clay loam	47.49	47.70	47.58	51.07	51.28	7.92
12	Cantaloupes	Sandy loam	39.93	37.69	38.81	20.60	20.74	7.36
13	Alfalfa	Clay loam	45.95	44.27	45.11	40.00	42.31	6.80
14	Orchard	Clay loam	48.68	46.79	47.73	52.75	51.91	16.04
15	Orchard	Sandy loam	48.69	49.46	49.07	48.25	53.31	15.86
16	Orchard	Sandy loam	47.00	47.21	47.10	52.40	52.89	13.73
17	Adobe hill	Raw ashobe clay	37.06	37.69	37.37	23.47	23.88	12.75
18	Beet field	Hard gravelly clay	28.09	27.95	28.02	39.72	39.86	2.94
19	Barley field	Clay loam	15.81	43.64	44.72	47.00	48.48	2.83
20	Beet field	Clay loam	48.20	48.27	48.23	39.44	38.53	13.38
21	Orchard	Clay loam	47.98	47.77	47.87	48.45	49.88	12.75
22	Orchard	Sandy loam	40.91	38.88	39.89	35.22	30.54	9.39
23	Orchard	Sandy loam	44.83	45.81	45.32	30.40	30.34	10.23
24	Orchard	Sand and gravel	17.77	48.82	48.29	38.25	39.86	11.56
25	Orchard	Coarse sand, almost gravel	17.49	47.42	47.45	35.38	32.71	16.81
26	Orchard	Sandy loam	50.72	50.99	50.85	44.69	44.83	19.05
27	Orchard	Clean sharp gravel	22.83	22.42	22.62	46.23	46.51	15.34
28	Orchard	Moist sand and gravel	38.39	38.67	38.53	46.79	46.65	15.48
29	Truck patch	River bottom sandy loam	20.32	21.86	21.99	25.08	25.08	26.41
30	Beet field	Clay loam	35.03	35.59	35.31	11.21	11.00	34.18
31	Beet field	Clay loam	27.53	29.42	28.47	21.30	19.83	35.52

\* Determination made after 11 days.

other samples were obtained, and the tract is 36 miles from the nearest recorded case of niter. The soil is a deep river bottom silt loam and is in a most productive condition, the owner having obtained 240 sacks of potatoes per acre in 1911. All kinds of vegetables, together with strawberries, are grown here very successfully. The soil has been heavily manured, and for that reason, I expected to find it abundantly stocked with all kinds of ammonifying bacteria. My expectatons were not fulfilled, however, for the ammonifying efficiency was less than any of the other soils examined and only about half as great as the general average for the niter soils. From cottonseed meal, 22.49% of the nitrogen was recovered as ammonia; dried blood 26.48%; alfalfa meal 2.13%; flaxseed meal 3.11%.

#### SAMPLES NOS. 30 AND 31.

These soils were taken from an entirely different part of the state than any of the others and come from a sugar beet field where very interesting soil conditions maintain. The tract, as a whole, is on high ground, but slopes rather rapidly from all sides into a hollow or basin near the center. This is wet and white with alkali much of the time. Between this part and the higher surrounding portion lies a zone which slopes gently toward the basin proper. Although p'anted to beets for two successive years, none have grown next to the white alkali at any time, and during 1911 none grew anywhere in this zone, not even at a considerable distance from the alkali, where a stand had been obtained in former years. The soil in this belt was brown, encrusted, and mealy, but not white. Immediately adjacent to the white alkali, it was wet and muddy, but the belt proper carried about the optimum amount of moisture for growing crops. The boundary of the white area appears to remain about the same from year to year, but the surrounding brown zone is moving gradually up the slope a little farther each year, the progress for 1911 having been at least 100 feet. With the advancing line of nitrates, the beets have been forced to recede, and each year the limit for their growth is set a little farther back. Sample No. 30 was collected from the brown, mealy niter zone where nothing grew, and No. 31 from that part of the field where there was a good

*Table No. 3. Nitrogen as Ammonia Originally Present in 100 Grams of Soil.*

Soil No.	Milligrams Nitrogen as Ammonia	Soil No.	Milligrams Nitrogen as Ammonia	Soil No.	Milligrams Nitrogen as Ammonia
1	.4203	11	.9807	21	.8406
2	.7005	12	.8406	22	.8406
3	.4203	13	1.1208	23	.2802
4	.2802	14	.1401	24	0.0000
5	.4203	15	.5604	25	.1401
6	.7005	16	1.2609	26	0.0000
7	.2802	17	0.0000	27	0.0000
8	.1401	18	2.6619	28	0.0000
9	1.2609	19	.7005	29	3.5025
10	1.1208	20	.9807	30	.4203
				31	1.2609

stand of beets, and where the soil, a clay loam, was normal to all appearances. Except for the low ammonification produced with the dried blood, No. 30 behaved much the same as other niter soils, while No. 31 was very similar to a normal soil.

The results follow:—No. 30, from cottonseed meal, 35.31% nitrogen was recovered as ammonia; dried blood 11.10%; alfalfa meal, 7.99%; flaxseed meal, 1.33%.

No. 31, from cottonseed meal, 28.47%; dried blood, 20.56%; alfalfa meal, 2.38%; flaxseed meal, 7.57%.

## DISCUSSION OF RESULTS.

### *Niter Soils and Normal Soils.*

A careful examination of the ammonia determinations given in Table No. 2 points very strongly to the niter soils as being superior to our normal soils in ammonifying efficiency. This becomes more apparent when typical soils are selected from each class, although this property is quite evident from the results as a whole.

While some ammonia may have resulted from a reduction of the nitrates present, as a matter of fact, I think that there is little ground for believing that this is the case. A number of these soils have been examined quantitatively for nitrates, and the amounts present are not sufficient to account for the ammonia formed. In the light of this fact, any hypothesis for the formation of ammonia based upon the reduction of nitrates appears to be without foundation.

Four soils are given in Table No. 4 which have never shown any indication of excessive nitrates either by a brown color or by injury to vegetation. The soils presented in Table No. 5 were all collected from areas where the niter is just now beginning to be very active in the destruction of trees. In this connection, let me emphasize this point, that these samples were not taken from old niter areas where everything had been killed, bacteria included, but they were obtained either from new localities, or, in case they did come from the sites of well established niter spots, from the margins of such areas where the accumulation of nitrates was in progress rather than completed.

*Table No. 4. Ammonifying Efficiency of Normal Soils.*

No.	Source	Character	Per cent. nitrogen recovered		as ammonia in 7 days from:	
			Cottonseed meal	Dried blood	Alfalfa meal	Flaxseed meal
3	Orchard	Sandy loam	25.92	18.03	12.06	6.30
10	Orchard	Clay loam	28.33	23.57	4.97	8.14
18	Beet Field	Gravelly clay	28.02	39.79	2.83	5.26
29	Truck patch	Silt loam	21.09	25.08	.73	3.11

*Table No. 5. Ammonifying Efficiency of Niter Soils.*

No.	Source	Character	Per cent. nitrogen recovered		as ammonia in 7 days from:	
			Cottonseed meal	Dried blood	Alfalfa meal	Flaxseed meal
11	Orchard	Clay loam	47.58	51.17	13.59	12.15
14	Orchard	Clay loam	47.73	52.23	16.56	3.99
16	Orchard	Sandy loam	47.10	52.64	13.69	.21
21	Orchard	Clay loam	47.87	49.16	12.22	.77
26	Orchard	Sandy loam	50.85	44.76	26.44	9.53

With cottonseed meal and dried blood, the ammonification has been almost twice as great in the niter soils as in the normal ones, while with alfalfa it has been from three to twelve times as much; the results from the flaxseed meal are so variable and irregular that any conclusions drawn from these figures would be little more than conjecture.

#### *Ammonification of Flaxseed Meal.*

In practically every culture that contained flaxseed meal, there was a heavy mycelial growth covering the entire substratum and filling the dish nearly to the cover, in some instances. This was especially noticeable with flaxseed meal, although occasionally a limited amount of a similar growth appeared in the presence of the alfalfa meal. What relation these fungi may have had to the relatively small amounts of ammonia recovered from the two substances mentioned is an open question. On this point Lipman (1) suggests the following:

"Is it because substances possessing a large proportion of non-nitrogenous compounds fail to undergo ammonification entirely or is it because the ammonia produced in the course of their decomposition is rapidly changed back into protein substances? As to the first assumption it is hardly in accord with facts now known \* \* \* \* It seems more likely that some ammonia was produced out of these materials, but on account of the relatively large supply of carbohydrates, molds and acid producing bacteria utilized the ammonia formed for the development of their body substances. In other words, whatever ammonia was produced, was utilized effectively for the development of mycelia and of bacterial cells. It seems reasonable to suppose, further, that the substances rich in protein favor the development of an alkaline reaction on account of the larger amounts of ammonia and ammonium carbonate formed. The alkaline reaction favors, in its turn, the vigorous growth of the more typical putrefactive organisms capable of causing fairly intense cleavage of protein compounds."

#### *Comparative Studies.*

In almost any investigation, a comparison of one's results with the work of others along similar lines leads either to a confirmation of truths, or to the discovery of new facts. Such a comparative study has been made between some of our soils and those from other localities in the United States, and the differences in ammonifying efficiency brought out in this way have been most striking as the figures given in Table No. 6 indicate. The methods employed by the different experimenters have been practically the same, so the results should be comparable. The two points which stand out most prominently in the tabulation of these results are:—First, in degree of ammonifying efficiency, the niter soils of Colo-

(1) Lipman, Jacob G., The Availability of Nitrogenous Materials as Measured by Ammonification. Cent. f. Bakt. Abt. II. Bd. 31, No. 1-4, p. 64, 65, 1911.

rado far exceed the soils from the other regions cited; second, the degree of ammonifying efficiency manifested by our normal soils is about the same as that of other soils, with a slight difference in favor of the Colorado samples.

The first four samples in Table No. 6 represent four localities in the state and three distinct types of soil where nitrates are making their presence manifest by injury to apple trees. No. 17 came from the top of an adobe hill, and is as nearly raw land as can be found in Colorado, in fact, it might be classified more correctly as a weathered shale than as soil. The last three were obtained from widely separated districts, and may be considered normal arable soils so far as the presence of excessive nitrates and crop yields are concerned. The soil numbers given in the above table correspond to the sample descriptions given in the preceding pages.

The character of New Jersey soil No. 1 is not recorded in the text from which I have secured the analysis, but No. 2 is given as a silt loam. Calcium carbonate was added to the latter soil along with the cottonseed meal and linseed meal to neutralize any organic acids that might be formed during ammonification. This may account for the close agreement between the New Jersey and Colorado results in the one case, since our soils contain an abundance of carbonate normally.

*Table No. 6. Ammonifying Efficiency of Colorado Soils Compared With Other Soils.*

Soil	Character	Per cent. nitrogen recovered		as ammonia in 7 days from:	
		Cottonseed meal	Dried blood	Alfalfa meal	Flaxseed meal
Colorado 2	Heavy clay	42.31	47.04	12.78	8.09
Colorado 8	Clay loam	51.98	47.98	15.30	1.12
Colorado 14	Clay loam	47.73	52.33	16.56	3.99
Colorado 16	Sandy loam	47.10	52.64	13.69	.21
Colorado 17	Raw adobe clay	37.37	23.67	12.75	2.87
Colorado 10	Sandy loam	28.33	23.57	4.97	8.14
Colorado 18	Gravelly clay	28.02	39.79	2.83	5.26
Colorado 29	River bot. silt	21.09	25.08	.73	3.11
New J. 1 (1)	Unknown	4.95	16.74	...	...
New J. 2 (2)	Silt loam, limed	41.18	56.63	...	46.06
Iowa (3)	Marshall loam	29.82	24.18	...	...
Calif. (4)	L. Sandy loam	...	18.99	...	...
N. Car. (5)	Unknown	31.86	...	...	...
No. 2069					
N. Car.	Unknown	22.06	...	...	...
No. 1931					

The California sample is described as a "light sandy loam free from alkali, from a walnut grove in Southern California—fairly well supplied with humus, owing to the careful system of green

(1). Marshall's Microbiology, p. 254. The ammonia determinations were made after 6 days.

(2). Lipman, Brown and Owen. The availability of nitrogenous materials as measured by ammonification. Cent. f. Bakt., Abt. II., Bd. 31, No. 1-4 p. 49, 1911.

(3). Brown, P. E. Some Bacteriological Effects of Liming. Research Bul. No. 2, Iowa Exp. Sta.

(4). Lipman Chas. B. Toxic Effects of "Alkali salts" in Soils on Soil Bacteria. I. Ammonification. Cent. f. Bakt., Abt. II., Bd. 32, No. 1-2, p. 58, 1911. Ammonia determinations made after 4 days.

(5). Stevens and Withers. Studies in Soil Bacteriology, II. Ammonification in soils and in solutions. Cent. f. Bakt., Abt. II., Bd. 23, No. 21-25 1909.

manuring which was practiced on it, and containing a vigorous flora ammonifying bacteria." The ammonia determinations with this soil were made after four days in place of seven as with the others.

The Iowa sample carries the following description:—"The soil was typical of the Wisconsin Drift, being classed by the Bureau of Soils as Marshall loam. It was obtained from an experimental plot to which no lime had ever been applied—which, during the preceding five years had been continually in corn and which prior to that time had been in a general farming rotation."

With the exception of the New Jersey figures, the percentages given in Table No. 6 are based upon blood meal containing 13.05 per cent. of total nitrogen, and cottonseed meal with 7.84 per cent. total nitrogen. In the New Jersey work, Lipman states that the blood meal and cottonseed meal used contained respectively 13.18 per cent. and 6.405 per cent. total nitrogen.

The California and Iowa samples fall considerably below the cultivated Colorado soils, containing nitrates, in ammonifying efficiency, although the figures for the former may be low on account of the four day experimental period in place of seven. New Jersey No. 1 appears to be greatly inferior to our soils, while No. 2 compares very favorably. It is interesting to note, in passing how much more available linseed meal seems to be with the limed New Jersey soil than with ours. While the former gives 46.06 per cent. nitrogen as ammonia, few Colorado soils will produce to exceed 13 per cent. and the majority less than 3 per cent.

## SUMMARY.

The power to transform organic nitrogen into ammonia is a property common to many cultivated Colorado soils.

Soils in the incipient stage of the niter trouble appear to surpass our normal soils in ammonifying efficiency.

Compared with soils from other localities, our niter soils excel in ammonifying efficiency to a very marked degree.

Nineteen of the thirty-one soils examined have ammonified cottonseed meal more readily than the other nitrogenous materials employed; the remaining twelve have broken down the dried blood most easily; twenty-six have formed ammonia from alfalfa meal more readily than from flaxseed meal, and with five the reverse has been true.

The maximum per cent. of ammonia produced in seven days by any soil from 100 m. g. of nitrogen as cottonseed meal was 51.98%; as dried blood 25.64%; as alfalfa meal 34.85%; as flaxseed meal 12.15%.

# ALGAE IN SOME COLORADO SOILS.

By W. W. ROBBINS.

## INTRODUCTION.

It has been experimentally demonstrated by Professor Walter G. Sackett (1) that many of the cultivated soils of Colorado possess the power to fix free atmospheric nitrogen. This fixation takes place in the soils themselves as well as in culture solutions. *Azotobacter chroococcum* is found to be the chief nitrogen fixing organism. It is now well known that unprecedented quantities of nitrates accumulate in certain soils of Colorado, resulting in so-called "niter areas"; the quantities are such as to kill off not only higher types of plants but the nitrogen fixing organisms themselves. The evidence brought to light by Dr. W. P. Headden (2), showing that the accumulation of these nitrates is not due to seepage or ground waters is too clear and certain to admit of dispute. Added to this are the results brought forward by Professor Sackett that certain of our soils have a high nitrogen fixing power. Naturally, the unusual accumulation of nitrates is thought to be due to the fixation of free atmospheric nitrogen by the soils themselves, accompanied by ammonification and nitrification.

As our soils are poor in organic matter, it seemed difficult to account for the source of energy that would be necessary to support such a rich nitrogen fixing flora. If it could be shown that our soils have an abundance of algae present, this condition would, at least, be highly suggestive that the energy for *Azotobacter* was being supplied in large part by these chlorophyl-bearing organisms. Hence it was that, with this in mind, the present preliminary study of the algae in our soils was undertaken.

I am indebted to Professor Sackett for the problem and for many laboratory facilities extended to me in the course of this study. The soil samples were collected by him.

## HISTORICAL.

It is well known that certain bacteria and algae enter into a symbiotic relationship, in which the latter furnish the bacteria with the necessary energy in the form of carbohydrates, while the bacteria supply the algae with nitrates. MM. Bouilhac and Guistiniana (3) showed that *Nostoc punctiforme* and *Anabaena*, when associated with bacteria, grew well on sand supplied with mineral nutrients in which nitrogen and organic material were lacking. Furthermore, the mixture could accumulate enough nitrogen to

(1) Sackett, Walter G., Bacteriological Studies of the Fixation of Nitrogen in Certain Colorado Soils. Colorado Agricultural Exp. Sta., Bull. 179, pp. 1-42, 1911.

(2) Headden, W. P. The Fixation of Nitrogen in Some Colorado Soils. Colorado Agricultural Exp. Sta., Bull. 155, pp. 1-48, 1910, and Bull. 178, pp. 1-96, 1911.

(3) MM. Bouilhac and Guistiniana. Sur une culture de sarrasin en présence d'un mélange d'algues et bactéries. Compt. rend. de l'Acad. T. CXXXVII, pp. 1274-1276, 1903. Sur des cultures de diverses plantes supérieures en présence d'un mélange d'algues et de bactéries. Comp. rend. de l'Acad. T. CXXXVIII, pp. 293-296, 1904.



enable certain higher plants to develop normally. Without algae, however, there was a comparatively slight growth of the higher plants. These experiments are not conclusive but they are indicative of the close relationship existing between algae and bacteria in the soil. More than that, they lead one to believe that in these experiments algae supplied carbohydrates for the nitrogen fixing bacteria which in turn furnished nitrates essential for the normal development of the higher plants.

Dr. Hugo Fisher (1) speaks of the symbiosis existing between *Azotobacter chroococcum* and *Oscillatoria*. He is of the opinion that *Azotobacter* occurs abundantly between the algal filaments, the algae furnishing carbohydrates, the bacteria nitrates. Frank (2) found that there was an increase in the nitrogen content of a nitrogen-poor sand on which algae developed in the light, while the same sand if kept in the dark did not increase in nitrogen. Soil bacteria were present in both cases. Schloesing and Laurent (3), working along the same lines, showed that soil containing both bacteria and algae could fix free nitrogen in large quantities while the same soil covered with quartz to prevent algal growth did not increase in nitrogen. The above workers assumed that the algae in their mixtures had the power to fix free nitrogen. This assumption was later proven to be erroneous, at least for green algae (Chlorophyceae).

In 1894, P. Kossowitsch (4), working with pure cultures of green algae, species of *Cystococcus* and *Stichococcus*, demonstrated that these alone could not assimilate the free nitrogen of the air. Later, in 1900, Kruger and Schneidewind (5), using pure cultures of green algae, species of *Stichococcus*, *Chlorella* and *Chlorothecium*, substantiated the results of Kossowitsch and proved that the green algae they used did not have the power to fix free atmospheric nitrogen. It is highly probable that none of the green algae possess this power. They further showed that when inorganic or organic nitrogen was excluded from the nutrient solution, all the species of algae in pure culture made no noticeable growth. There was abundant growth, however, when the same sub-stratum was supplied with combined nitrogen. In neither of the above pure cultures of algae was there any nitrogen fixation. But in the same medium, both an abundant development of algae and a fixation of nitrogen took place if pure cultures of the algae were inoculated with *Azotobacter*. In the latter case fixation is, of course, attributed to *Azotobacter*, while algae furnished them with the organic matter necessary for their life.

(1) Fisher, H., Ueber Symboise von *Azotobakter* mit *Oscillarien*. Cent. f. Bakt. Abt. II., Bd. XII., p. 267, 1904.

(2) Frank, A. B., Ueber den experimentellen Nachweis der Assimilation freien Stickstoffes durch erdbewohnende Algen. Berichte d. deutschen botan. Gesellschaft. Bd. VII., p. 5, 1889.

(3) Schloesing and Laurent, Recherches sur la fixation de l'azote libre par les plantes, Annales de l'Institut Pasteur T. VI., p. 65 W. 824, 1892.

(4) Kossowitsch, P., Untersuchungen ueber die Frage, ob die Algen freien Stickstoff fixieren. Botan. Zeitg., Bd. LII, p. 97, 1894.

(5) Kruger and Schneidewind. Sind niedere, chlorophyllgrüne Algen imstande, den freien Stickstoff zu assimilieren? Landwirtschaft. Jahrb. Bd. XXIX., p. 771 ff., 1900.

Attempts to secure pure cultures of blue-green algae (Cyanophyceae) have been attended with failure and hence there are no reliable experiments which prove that these possess the ability to assimilate free nitrogen. Our further studies will be directed to clearing up the relations of the blue-green algae to *Azotobacter chroococcum* and nitrogen fixation. It is a significant fact that the blue-greens are by far the most abundant algae in all Colorado soils examined.

### METHODS.

In each case the samples included the surface 3 to 4 inches of soil. Any debris on the surface was first removed. The samples were taken during October, 1911, and brought from the field to the laboratory in sterilized, double, sugar sacks. In the laboratory, the soil was transferred to sterilized Mason jars.

Florence flasks, 500 c. c. capacity, were filled to their greatest diameter with ground quartz which was previously washed free from all suspended matter. These were sterilized in an autoclave for 30 minutes at 120° C., in a moist condition. Each flask was plugged with sterile cotton. After removing flasks from the autoclave they were so placed as to get a smooth, horizontal surface of the substratum.

For inoculation 20 g. of each soil sample were shaken up for 5 minutes in 50 c. c. of sterile, distilled water. An amount of suspension material corresponding to 10 g. of soil, i. e. 25 c. c., was drawn off with a sterile pipette and distributed as evenly as possible over the ground quartz surface.

With the above precautions, contamination was impossible; the abundant algal growth which appeared in all but two flasks assuredly represents only those forms existing in the soils used for inoculation. Of course, it is quite well known that sterile, distilled water is essential; tap water may carry both vegetative and reproductive parts of algae.

The flasks, 22 in number, containing as many different soil samples, were placed in the greenhouse in a sunny place. Later they were removed to a shady situation in the botanical laboratory where they grew fully as well as in the greenhouse. Each flask was tipped to one side so as to offer both a moist sand and a free water surface for the algae to grow on.

A number of species of algae which appeared in the flask cultures were transferred to a 1% agar medium in which soil extract was used as the nutrient solution. The soil extract was prepared by filtering soil in Pasteur-Chamberland, unglazed porcelain filters. In preparing Petri dish cultures, a small bit of algal material was removed from the flasks and shaken up vigorously in a test tube with about 2 c. c. of distilled water. Platinum wire loops from this were transferred to tubes of liquid 1% agar at 42° C. These were shaken and then poured into Petri dishes. Algal growth never

failed to appear in these agar cultures and in many cases within 2 or 3 weeks. In some instances subsequent reinoculations to agar were made from the Petri dishes.

### DESCRIPTIONS OF SOIL ALGAE.

Hereinafter is included a brief description of each algal species found in the samples of soil examined. Although most of these species are described in the books on algae (1), it seemed desirable to give here complete descriptions and illustrations of all the species found in the soils examined. The reader will thus gain a better idea of the nature of the algal forms which, up to date, have been found in our soils. Some of the descriptions are taken from Tilden's *Minnesota Algae*. Credit is due Miss Nellie Killgore who made most of the drawings and both colored plates.

It will be noted that with but two exceptions, all the species found in the soil samples belong to the blue-green algae (Cyanophyceae). It will be recalled that the blue-greens include the simplest kinds of algae. They are characterized by simple asexual methods of reproduction and by the presence of a blue pigment, phycocyanin, in addition to a green pigment; the mixture results in a blue-green color. The plant body may be unicellular or multicellular. Unicellular forms may be single or grouped into colonies; multicellular species are mostly filamentous. It is worthy of note that the blue-greens found in the soils examined are all filamentous. Furthermore the largest proportion of them belong to the one family Nostocaceae. This family includes members usually possessing thick, gelatinous or mucous sheaths surrounding the trichomes, or rows of cells. Other families of blue-green, (Oscillatoriaceae, Stigonemaceae and Rivulariaceae) represented in the soil, also have gelatinous coverings to the trichomes. I mention the fact that forms of algae which have gelatinous sheaths predominate here, because I believe that bacteria find in these sheaths a highly favorable nutritive medium. Kossowitsch and Schloesing and Laurent observed that in their cultures which showed nitrogen fixation in great amounts, *Nostoc*, a blue-green alga with gelatinous sheaths, was the dominant form present. Again, the presence of this sheath probably accounts for the difficulty experienced in attempting to get such algae in pure cultures, free from their accompanying bacterial and fungal flora. The gelatinous coatings undoubtedly harbor a host of bacteria. European investigators have experienced but comparatively little difficulty in getting the unicellular green algae in pure cultures. On the other hand, I find no recorded instance of pure cultures of such forms as *Nostoc*. Green algae do not as a rule have such thick coverings of gelatinous material as members of the blue-greens.

We have succeeded in getting the green alga so abundant in Sample 7 practically pure, while our efforts in this regard with blue-greens

(1) The writer has made most use of Tilden's *Minnesota Algae* and De Toni's *Sylloge Algarum*, vol. 5, the Myxophyceae.

are unsuccessful. We hope, however, to overcome the difficulties and obtain absolutely pure cultures of the most dominant blue-greens occurring in our soils. With such pure cultures we will be in a position to test their supposed nitrogen fixing power, and their role in the soil.

A number of the species appear to be undescribed. Although it has been possible to follow these for some months with considerable care and satisfaction through to spore production and growing both in flasks on sterile ground quartz and in 1% aqueous agar, these will, for the present, be designated by letters until further study of them is made. It has been impossible to identify certain other forms on account of their immaturity. For example *Stigonema* and *Rizularia* specimens were in developmental stages.

It was anticipated that the systematic study of algae occurring on and in the surface layers of soil would be attended with difficulty. This is largely due to the fact that no previous studies of soil algae have, to our knowledge, been made; furthermore, the descriptions of many species are totally inadequate and undifferentiating. It is needless to say that a systematic study of these soil organisms is highly essential. It is our purpose to continue the systematic study of the algal flora of Colorado soils as well as its relation to nitrogen fixation.

**Oscillatoria formosa** Bory. Plate I., fig. 1. Soil capillarity tube. Plant mass dark blue green; trichomes straight, elongate, usually slightly constricted at joints; apex of trichome somewhat obtuse and briefly tapering or rotund, hooked, not capitate; calyptra none; cells 2.5-5 mic. long; transverse walls finely granulate; cell contents bright blue-green.

**Phormidium inundatum** Kuetzing. Plate I., fig. 2. Soil samples 9, 10, and soil capillarity tube. Filaments somewhat straight, fragile; scattered in the flask cultures among other algae; sheaths thin; trichomes 3-5 mic. in diameter, straight or curved, not constricted at joints; apex of trichome straight, briefly tapering, not capitate; apical cell obtuse conical; calyptra none; cells 4-8 mic. in length; transverse walls covered with protoplasmic granules.

**Phormidium subuliforme** Gomont. Plate I., fig. 3. Soil sample 10. Filaments scattered throughout other algae; trichomes 2-2.8 mic. in diameter, straight, constricted at joints; apex of trichome gradually tapering, bent or twisted, not capitate; apical cell more or less acute-conical; calyptra none; cells 6-8 mic. in length; transverse walls indistinct; cell contents homogeneous or coarsely granular, blue-green.

**Phormidium tenue** (Meneghini) Gomont. Plate I., fig. 4. Soil samples 4, 10, 11, 16, 18 and soil capillarity tube. Plant mass thin, membranous, expanded, pale blue-green; filaments elongate, straight, entangled; sheaths thin; trichomes 1-2 mic. in diameter, straight, somewhat constricted at joints; apex of trichome at first straight becoming tapering and bent; cells 2.5-5 mic. in length; transverse walls usually indistinct.

**Phormidium valderianum** (Delponte) Gomont. Plate I., fig. 5. Soil samples 13, 15. Filaments flexuose, densely entangled, here scattered throughout other algae; trichomes 2-2.5 mic. in diameter, straight not constricted at joints, apex of trichome not tapering; apical cell rotund; calyptra none; cells 3.3-6.7 mic. in length; transverse walls marked by two or four protoplasmic granules; cell contents blue-green.

**Microcoleus vaginatus** (Vaucher) Gomont. Plate I., figs. 6, 7. Soil capillarity tube. Filaments forming entangled and twisted threads, dark

olive or black in color; sheaths cylindrical, more or less unequal in outline, agglutinated, pointed and closed at the apex, or open and gradually disappearing, at times entirely diffuent; trichomes 3.5-7 mic. in diameter, not constricted at joints, many within the sheath, closely crowded, usually twisted into cords, the portion extending from the sheath straight; apex of trichome gradually tapering and capitate; outer membrane of apical cell thickened into a depressed conical calyptra; cells 3-7 mic. in length; transverse walls frequently granulated.

**Nostoc "A".** Plate III., figs. 3, 4. Soil samples 1, 10, 11, 13, 21. Plant mass gelatinous, irregularly expanded, at first bright blue-green, becoming light olive or pale pea-green; filaments mostly straight sometimes loosely entangled or rarely spirally rolled; sheaths colorless, indistinct, becoming confluent; trichomes 5.2-6.7 mic. in diameter; cells different in shape, usually short depressed-spherical or barrel-shaped, 3.9-6.2 mic. in length, at first bright blue-green becoming grayish-green, the granules large and conspicuous; heterocysts yellowish-green, spherical, spherical-depressed or a little longer than wide, 6-8.3 mic. in diameter, 6-8 mic. in length; gonidia 6-8 mic. in diameter, 8-13 mic. in length, oval to oblong, separated, often irregularly disposed, grayish-green; wall of gonidium smooth, colorless. Habitat: cultivated soil.

A small form of the above species (Plate I., fig. 8) occurs in samples 11 and 21. Trichomes 3.6-4.7 mic. in diameter; heterocysts 4.3-5.9 mic. in diameter; gonidia 5.2-6.7 mic. in diameter, 7.8-11 mic. in length.

**Nostoc "B".** Plate III., figs. 5, 6, 7, 8, 9. Soil samples 1, 2, 4, 6, 8, 10, 11, 15, 16, 20. Plant mass bluish-white becoming yellowish with age, shapeless; filaments 15-20 mic. in diameter, flexuous, entangled, pale blue-green; trichomes 3-4.5 mic. in diameter, single in each colorless sheath; cells barrel-shaped or cylindrical, 3-6.5 mic. in length; heterocysts globose to elongate, 3.5-5.5 mic. in diameter, 5-5.2 mic. in length; gonidia numerous, separate, spherical to oval, brownish, 4.5-5.5 mic. in diameter 5-6.5 mic. in length; walls smooth. Habitat: cultivated soil.

**Nostoc "C".** Plate III., figs. 10, 11. Soil sample 4. Plant mass gelatinous-membranaceous, bright olive or dark colored; filaments flexuous, entangled; trichomes 3.6-4 mic. in diameter; cells depressed-spherical, barrel-shaped or ellipsoidal, blue-green, 3.6-5.7 mic. in length; heterocysts subglobose or oblong 5-5.2 mic. in diameter; gonidia oval, in long series, 5.2-6.2 mic. in diameter, 7.8-9 mic. in length; wall of gonidium smooth, deep amber. Habitat: cultivated soil.

**Nostoc commune** Vaucher. Plate I., fig. 13. Soil samples 1, 2, 5, 8, 11, 15, 16. Plant mass gelatinous, not assuming here any definite form; filaments flexuous, entangled; sheaths colorless or brownish; trichomes 4.5-6 mic. in diameter; (In samples 1 and 2, the trichomes are smaller, measuring 4-5 mic. in diameter) cells depressed-spherical or barrel-shaped; heterocysts 5.7-7 mic. in diameter, somewhat spherical; gonidia unknown.

**Anabaena "A".** Plate IV., figs. 6, 7, 8. Soil samples 1, 2, 5, 10, 13, 14, 18. Plant mass gelatinous, dark green; trichomes 2.8-4 mic. in diameter, straight or flexuous; cells barrel-shaped, 3.9-5.2 mic. in length; heterocysts spherical to ovoid, 4-5 mic. in diameter, 4.6-5.4 mic. in length; gonidia ovoid when young, becoming cylindrical, solitary or in series contiguous to heterocysts, 5-6 mic. in diameter, 10-18 mic. in length; wall of gonidium smooth, colorless. Habitat: cultivated soil.

**Nodularia armoricæ** Thuret. Plate I., figs. 9, 10. Soil sample 21. Filaments 10-11 mic. in diameter, entangled; sheaths thin; cells depressed, one-half as long as diameter; heterocysts compressed, somewhat larger than the cell; gonidia depressed-spherical, yellowish-brown, in series, 10-12 mic. in diameter, 9 mic. in length; end walls of gonidia transversely truncate, projected.

3-15 mic. in diameter, yellow green, often changing to shades of red by exposure. This is the common green alga found everywhere on soil, moist rocks, walls, trunks of trees, etc. In the soil capillarity tube, the orange yellow or reddish brown spots are due to this species.

*Navicula* sp. (Diatom) Plate II., fig. 18. Soil sample 19. Plants brownish, boat-shaped, bivalved, the valves marked by fine, parallel striations; individuals 4.8-5.2 mic. in diameter, 28-68 mic. long.

## DESCRIPTIONS OF FLASK CULTURES.

Here follows a brief description of the general nature of the algal growth and an enumeration of the species occurring in each flask. All flasks were inoculated between November 25 and 28, 1911. In some cases a slight green tinge to the water or quartz surface appeared within one month after inoculation. In most cases, however, no growth was apparent until the first part of February, 1912. The abundant development of the algae in sample No. 1 is typical of the majority of cases. Plate IV., fig. 10 is a water color drawing of this flask culture.

**Sample No. 1.—SOIL:** heavy clay, orchard. **GROWTH:** vigorous, covering the surface of the sand with a dark green coating and extending several centimeters above and below the sand surface on the sides of the flask. In places the growth is brownish-black, due to *Stigonema*. **ALGAE:** *Nostoc* "A", *Nostoc* "B", *Nostoc commune*, *Anabaena* "A", *Stigonema* sp.

**Sample No. 2.—SOIL:** sandy loam, orchard. **GROWTH:** at first blue-green, becoming grayish-green or yellowish in color; covering quartz and water surface and sides of flask. **ALGAE:** *Nostoc* "B", *Nostoc commune*, *Anabaena* "A", *Rivularia* "A".

**Sample No. 3.—SOIL:** sandy loam, orchard. **GROWTH:** none.

**Sample No. 4.—SOIL:** sandy loam, orchard; **GROWTH:** substratum covered with a yellowish-green scum. **ALGAE:** *Phormidium tenue*, *Nostoc* "B", *Nostoc* "C".

**Sample No. 5.—SOIL:** sandy loam, orchard. **GROWTH:** substratum wholly covered with a dark green mass; algae also extending for some distance below the sand surface along the sides of the flask. **ALGAE:** *Nostoc commune*, *Anabaena* "A".

**Sample No. 6.—SOIL:** clay loam, orchard. **GROWTH:** substratum entirely overgrown, at first blue-green, becoming yellowish-green. **ALGAE:** *Nostoc* "B".

**Sample No. 7.—SOIL:** sandy loam, orchard. **GROWTH:** the first evidence of algal growth appeared in this sample; this was one month after inoculation and was due to the unicellular green alga. The scum occurred on the quartz, water and sides of flask. **ALGAE:** *Rivularia* "A" *Stigonema* sp., unicellular green alga.

**Sample No. 8.—SOIL:** clay loam, orchard. **GROWTH:** scanty; scum thin. **ALGAE:** *Nostoc* "B", *Nostoc commune*.

**Sample No. 9.—SOIL:** clay loam, orchard. **GROWTH:** substratum covered with a gray-green scum; algae also extending upon the sides of the flask. **ALGAE:** *Phormidium inundatum*, *Nodularia harveyana*, *Nodularia* "A", *Stigonema* sp.

**Sample No. 10.—SOIL:** clay loam, orchard. **GROWTH:** plant mass blue-green covering the entire substratum. **ALGAE:** *Phormidium inundatum*, *Phormidium subuliforme*, *Phormidium tenue*, *Nostoc* "A", *Nostoc* "B", *Anabaena* "A", *Navicula*.

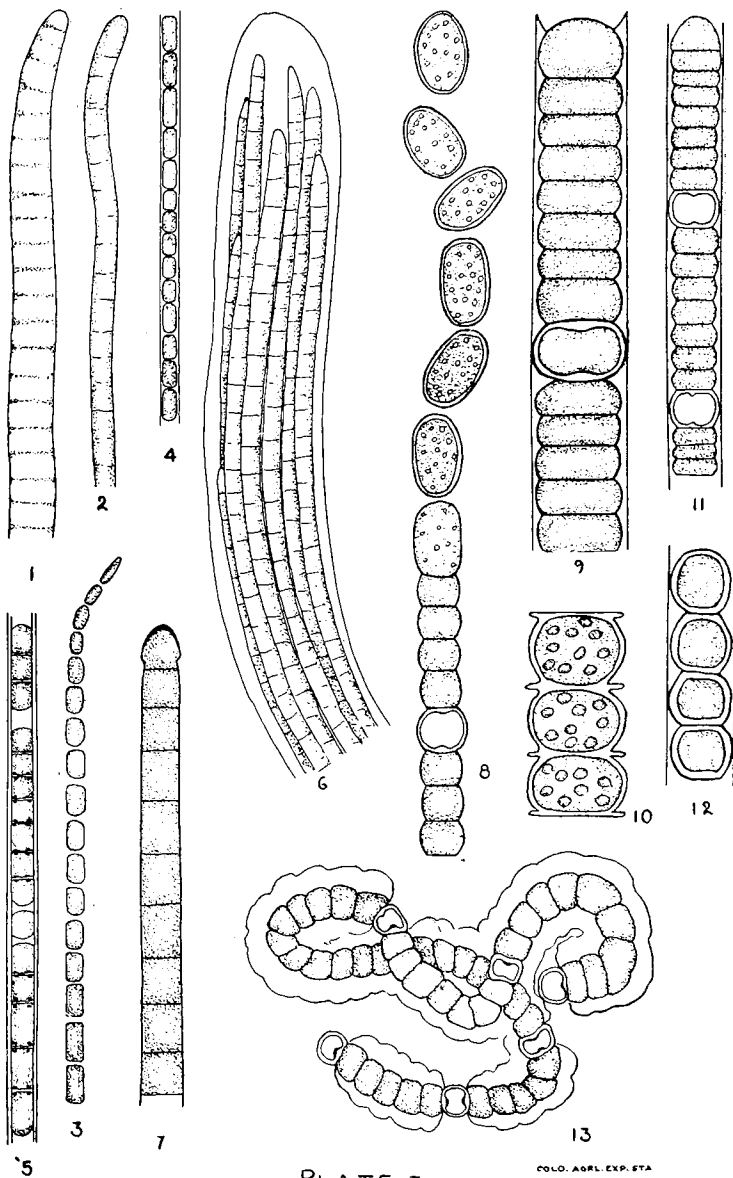


PLATE I

COLO. AGR. EXP. STA.





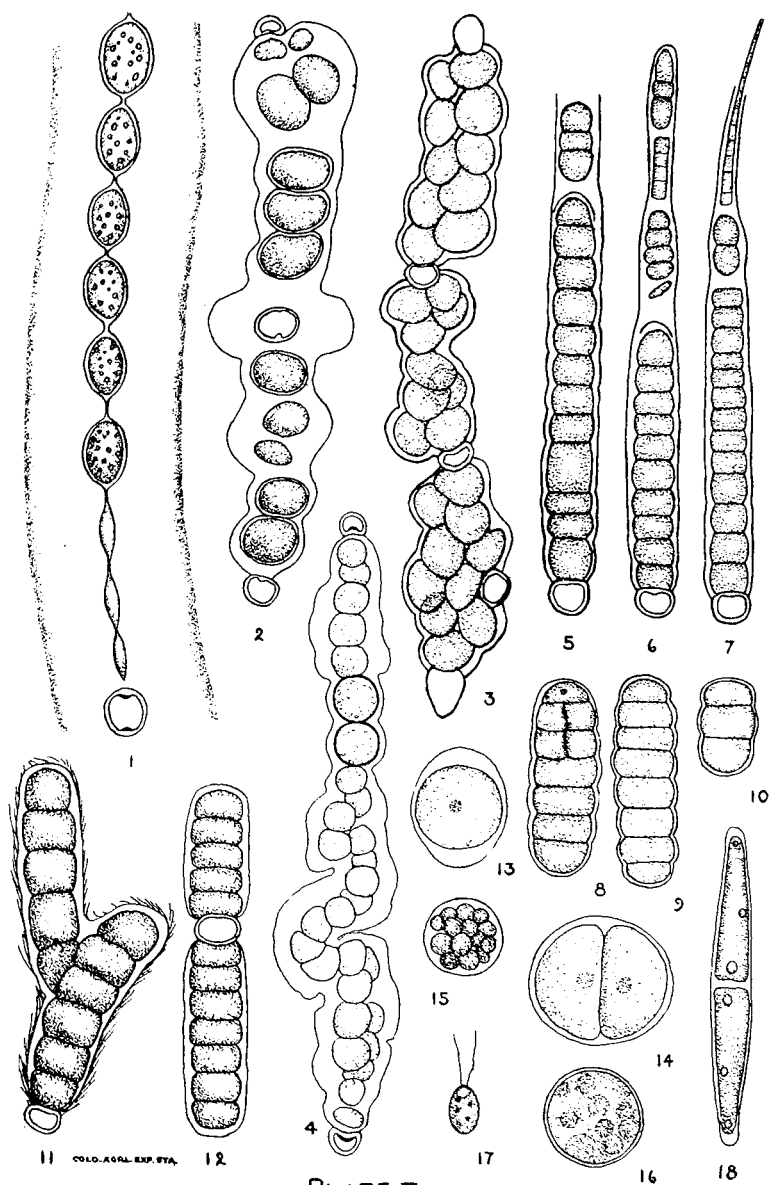


PLATE II.



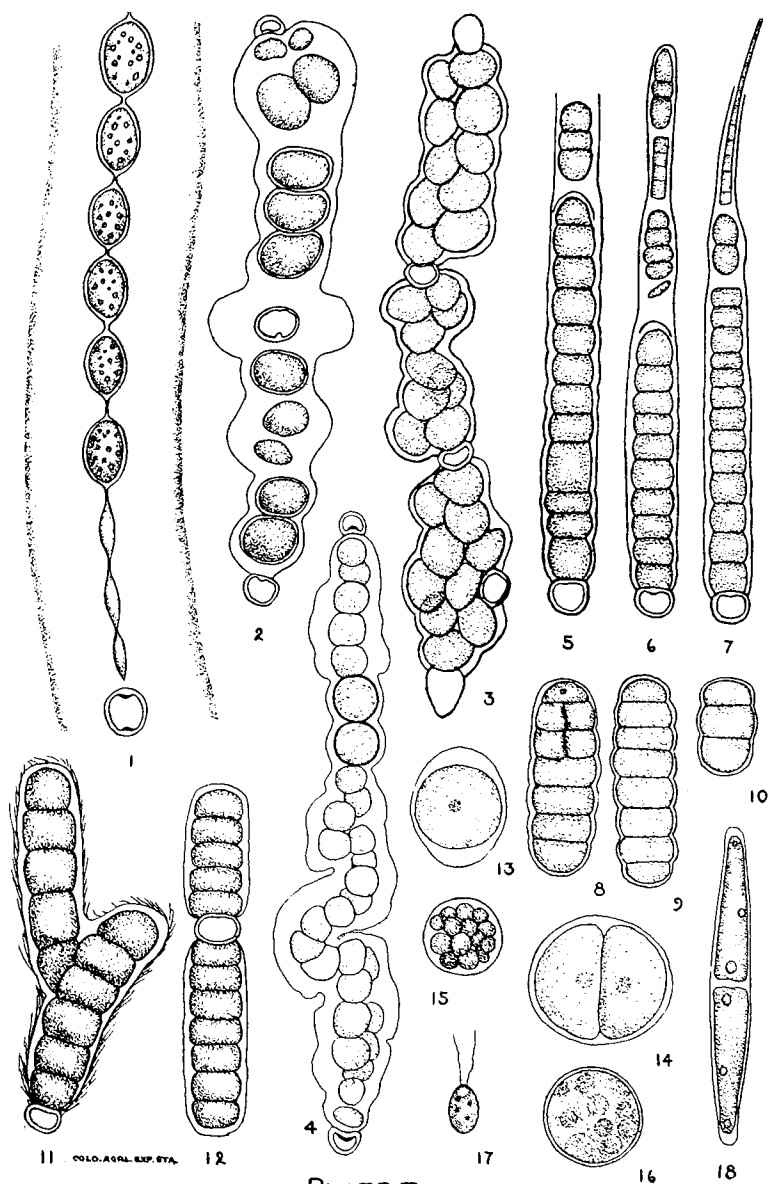
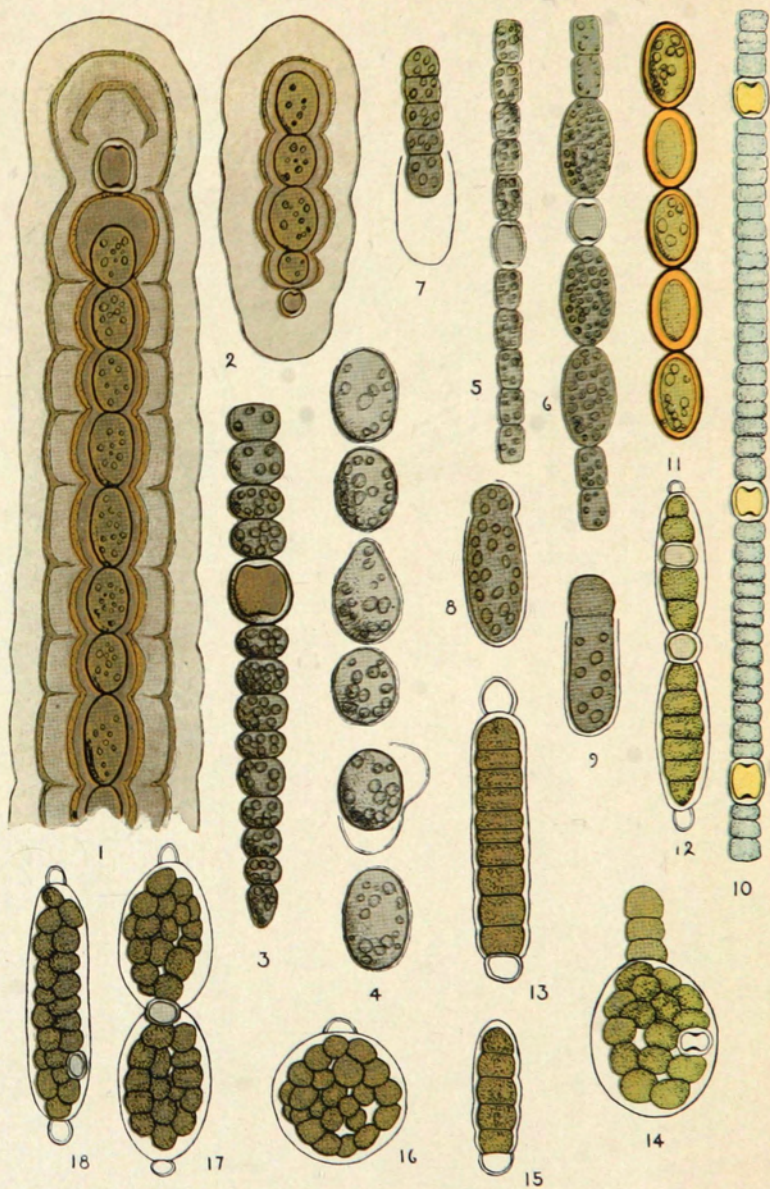


PLATE II.





CALIF. AGRI. EXP. STA.

# PLATE III.

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1

COLORADO AGRICULTURAL EXPERIMENT STATION



2



3



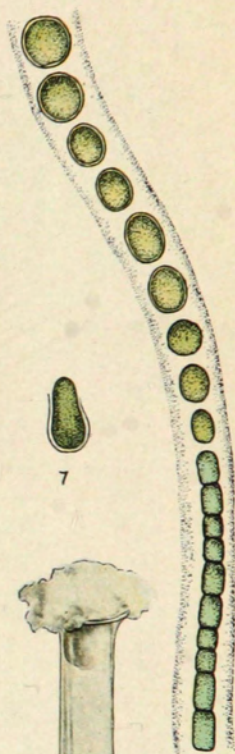
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5

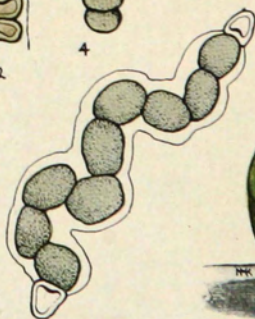


6

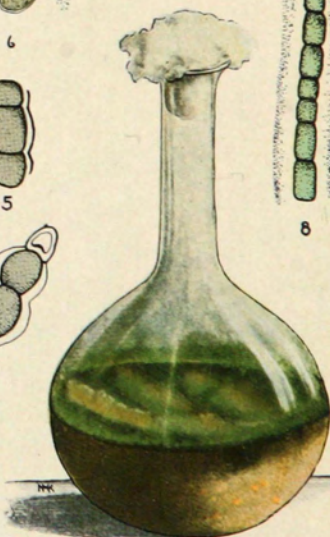


7

8



9



10

MELVIE M. KILLBORE

PLATE IV.





**Nodularia harveyana** (Thwaites) Thuret. Plate I., figs. 11, 12. Soil samples 9, 13, 14, 15, 16, 21, 22. Filaments long and straight, 6 mic. in diameter; sheaths thin, colorless, distinct; cells 5.2 mic. in diameter, 1.5-3.9 mic. in length; heterocysts depressed, 5.2-5.4 mic. in diameter, 4.6-5.2 mic. in length; gonidia in long series between the heterocysts, 6.5-8 mic. in diameter, 5.2-7.8 mic. in length, yellowish-brown.

**Nodularia "A"**. Plate IV., figs. 2, 3, 4, 5. Soil sample 9. Filaments long and straight, 7-8.5 mic. in diameter; sheath colorless, distinct; cells disc-shaped 5.2-7.5 mic. in diameter, 1.5-2.5 mic. in length; heterocysts depressed, mostly occurring in pairs, yellowish-green, 7.1-7.8 mic. in diameter, 4.4-5.6 mic. in length; gonidia spherical-depressed or spherical, brownish, 7.2-8.3 mic. in diameter, 5.4-7.7 mic. in length; wall of gonidium smooth. Habitat: cultivated soil.

**Stigonema "A"**. Plate II., fig. 1, and Plate III., figs. 1, 2. Soil sample 16. Plant mass rust colored; filaments 20-46 mic. in diameter; sheath lamellose, constricted at joints, the outermost layers colorless, the inner ones yellowish or yellowish-brown, with a special envelop about each cell; trichomes single within each sheath; heterocysts terminal or intercalary, yellowish or orange-colored, 3.6-5 mic. in diameter, 5 mic. in length, oval or pear-shaped; cells spherical, oval, oblong or cylindrical, 4-5.2 mic. in diameter, 5.2-10 mic. in length, often attenuated; apical cell elongate, conical; gonidia (?) oval, the ends slightly attenuate, greenish brown, 5-5.7 mic. in diameter, 7-10 mic. in length; wall of gonidium smooth.

**Stigonema** sp. Here are grouped a number of polymorphic, transition forms of what appear to be one or more species of **Stigonema**. These forms are very abundant in the samples but the stages of development are not such as to permit one to come to any definite conclusions as to their identity. They are described and figured here as a record of algae found in cultivated soils. Soil samples 14, 22. Plate II., fig. 3, and Plate IV., fig. 9. Trichomes contorted, in sac-like gelatinous, colorless envelopes; cells irregular in shape 5.2-6.5 mic. in diameter; heterocysts terminal or intercalary, 5.2 mic. in diameter. Soil sample 22. Plate II., fig. 2. Probably a gonidial stage of the above. Sheath inflated at the ends; heterocysts terminal or intercalary; gonidia brownish, 6.5-7.8 mic. in diameter, 5.2 mic. in length; wall of gonidium smooth. Soil sample 21. Plate II., fig. 4. Numerous elongated colonies in which the trichomes are highly contorted; cells irregular in shape, 4.6-6.5 mic. in diameter. Soil samples 7, 18, 20. Plate III., figs. 13, 14, 15, 16, 17, 18. Numerous spherical and oblong colonies of many sizes; cells 3.8-4.2 mic. in diameter, irregular in shape. Soil samples 1, 9. Plate II., fig. 4. Floating dark-brown crust; cells 5.2 mic. in diameter, irregular in shape; heterocysts 3.3 mic. in diameter, terminal or intercalary, some of them becoming thick-walled and granular.

**Rivularia "A"**. Plate II., figs. 5, 6, 7, 8, 9, 10. Soil samples 2, 7, 18, 20. Numerous developmental stages are present in the above samples. Filaments 7-10 mic. in diameter tapering; sheaths colorless, close; basal cells 5.2-6 mic. in diameter, 2.6-5 mic. in length.

**Rivularia "B"**. Plate II., figs. 11, 12. Soil sample 16. Filaments scattered in the sample; filaments branched; sheaths thin, ragged along the edges; basal cells 8-8.4 mic. in diameter, shorter than wide; heterocysts hemispherical, yellowish-green, 7.5 mic. in diameter.

**Unicellular green alga**. Plate II., figs. 15, 16, 17. Soil samples 7, 19. Plant mass bright yellow green; both motile and resting bodies present; motile individuals elongate, 3.3-5.2 mic. in diameter, 5-11 mic. in length; flagella 2 in number at anterior end, slightly longer than body; resting bodies spherical, varying much in size, usually 10-12 mic. in diameter.

**Pleurococcus vulgaris** Meneghini, Plate II., figs. 13, 14. soil capillarity tube. Unicellular, spherical forms, single or gathered into clusters. Cells

**Sample No. 11.—SOIL:** clay loam, orchard. **GROWTH:** thick, dark blue-green scum covering the substratum and extending up the sides of flask; the growth on the quartz surface is tuberculate. **ALGAE:** *Phormidium tenue*, *Nostoc* "A", *Nostoc* "B", *Nostoc commune*.

**Sample No. 12.—SOIL:** sandy loam, garden patch. **GROWTH:** unfortunately this flask was broken before identifications of the algae were made; the plant mass extended completely over the substratum.

**Sample No. 13.—SOIL:** clay loam, alfalfa field. **GROWTH:** an ample development irregularly expanded, forming a thick coating on the substratum. **ALGAE:** *Phormidium valderianum*, *Nostoc* "A", *Anabaena*, "A", *Nodularia harveyana*.

**Sample No. 14.—SOIL:** clay loam, orchard. **GROWTH:** plant mass spreading on the quartz and sides of flask as a thick, dark-blue, gelatinous mass. **ALGAE:** *Nostoc* "A", *Anabaena* "A", *Nodularia harveyana*, *Stigonema* sp.

**Sample No. 15.—SOIL:** sandy loam, orchard. **GROWTH:** abundant, blue-green, becoming yellowish-green due to the formation of gonidia. The minute dark green colonies between the quartz and sides of flask some distance below the surface are colonies of *Nostoc* "B". **ALGAE:** *Phormidium valderianum*, *Nostoc* "B", *Nostoc commune*, *Nodularia harveyana*.

**Sample No. 16.—SOIL:** red, sandy loam, orchard. **GROWTH:** quartz, water surface and sides of flask grown over with a blue-green gelatinous scum, becoming rust colored. **ALGAE:** *Phormidium tenue*, *Nostoc* "B", *Nostoc commune*, *Nodularia harveyana*, *Rivularia* "B", *Stigonema* "A".

**Sample No. 17.—SOIL:** raw soil, adobe hill. **GROWTH:** none.

**Sample No. 18.—SOIL:** hard, gravelly clay, beet field. **GROWTH:** light, blue-green scum covering the quartz surface. **ALGAE:** *Phormidium tenue*, *Anabaena* "A", *Rivularia* "A", *Stigonema* sp.

**Sample No. 19.—SOIL:** clay loam, beet field. **GROWTH:** about the second month after inoculation a slight green growth became evident, which later disappeared entirely. **ALGAE:** unicellular green alga.

**Sample No. 20.—SOIL:** clay loam, orchard. **GROWTH:** plant mass forming a thick, gelatinous layer over the whole substratum. Several darker or olive green masses here and there prove to be *Stigonema*. **ALGAE:** *Nostoc* "B", *Rivularia* "A", *Stigonema* sp.

**Sample No. 21.—SOIL:** sandy loam, orchard. **GROWTH:** the algal growth in this flask is the most vigorous of all. The sand, surface of water and sides of glass on all sides up to the neck of the flask are coated with a thick, mucous layer which was at first bright blue-green, but later became pale blue-green. This abundant development is due largely to *Nostoc* "A". **ALGAE:** *Nostoc* "A", *Nostoc* "B", *Nodularia armoricola*, *Nodularia harveyana*, *Stigonema* sp.

**Sample No. 22.—SOIL:** river bottom silt, truck patch. **GROWTH:** substratum grown over with a dark, brownish-green mass. **ALGAE:** *Nodularia harveyana*, *Stigonema* sp.

It is difficult to see, from the limited number of samples examined, any relation between soil type and abundance of algal development. Algae were found to be present in a variety of soils, for example, sandy loam, clay loam, heavy clay, hard, gravelly clay, heavy adobe and river bottom silt. While in samples Nos. 8 and 19, a clay loam, there was slight growth, in samples Nos. 6, 11, 13, 14, and 20, all of similar kind of soil, the development was vigorous, in most instances totally covering the substratum in the



flask. No algae appeared in sample No. 17, a raw soil from an adobe hill. Lack of moisture is undoubtedly an unfavorable factor in this case. In this connection, it should be said that cultivation, resulting in better aeration of the soil, is unquestionably favorable to increased activities of soil algae as well as other soil organisms. I can ascribe no reason for the non-occurrence or non-development of algae in Sample No. 3, a sandy loam from an orchard. It must be understood that the conditions under which all samples were grown were similar. It will be seen from Table I that the most prevalent species of algae in the 22 soil samples are *Phormidium tenue*, *Nostoc* "A", *Nostoc* "B", *Nostoc commune*, *Anabaena* "A", *Nodularia harveyana* and *Stigonema* sp. These not only occur in a greater number of samples, but they form, as a rule, the greater portion of the algal mass in the flasks.

### DESCRIPTION OF SOIL CAPILLARITY TUBE.

Mr. V. M. Cone, irrigation engineer in this Station, called our attention to the appearance of an abundant growth of algae in some soil tubes used in testing capillarity. One of these was chosen for examination of the algae it contained.

The soil, a sandy loam, had been replaced foot for foot in a tube, 1 inch in diameter, making a 5-foot column. The tube was placed in the laboratory about one foot from a wall, hence the algae grew only on the lighted side. Unfortunately, no data were secured as to the date of appearance of the algae, although it is known that development was first conspicuous in the third foot of soil. Finally, the first two feet exhibited the greatest development. There was algal growth, however, in every foot of soil except the fifth. It is not to be understood by this that algae grow at a depth of 4 feet or even 1 foot below the soil surface. It is very probable that surface waters continually carry spores from upper to lower soil layers: there the spores remain quiescent for a short period, finally dying unless favorable conditions are restored either by natural or artificial means. Extreme precautions are necessary in taking samples to prevent contamination of one soil layer with another. Again, it is essential that the tubes or vessels containing the soil, be previously sterilized: dust sticking to the sides may be a possible source of contamination. By a glance at the soil capillarity tubes showing algae growing in the first four feet, one might gain the notion that they grew at such depths under field conditions. Yet when the above possible sources of contamination of the lower soil layers are considered, the appearance of algae in the lower layers of the tubes in question, loses its significance.

The algae occur in the capillarity tube in patches ranging in size from mere specks to areas one or more inches in diameter. (Plate IV., fig. 1). The patches are, for the most part, irregularly circular in outline. The yellowish-green to reddish areas are *Pleurococcus*; the dark-green areas are mostly *Oscillatoria formosa*

and *Phormidium tenue*; the olive-colored colonies are *Phormidium tenue*. Here and there are interlacing masses of dark-olive or black threads, visible to the naked eye; these are *Microcoleus vaginatus*.

### DISCUSSION.

It is well known that many different kinds of algae inhabit the soil. As a rule, it is generally understood that such a soil is necessarily muddy or very moist. In such cases the algal growth is visible to the naked eye, forming on the soil a characteristic plant mass. The soils from which the foregoing 22 samples were taken were, with the exception of No. 17, just ordinary cultivated soils, with a varying water content. The samples were representative of soils in rather widely separated localities in Colorado. At the time of collection, during October, 1911, no algae were noticeable on the soil surface; furthermore, one would not ordinarily think of such soils as being moist enough to support an algal flora. And yet, cultures from these soils, with but two exceptions, samples Nos. 3 and 17, revealed the presence in them of a considerable number of species of algae and a healthy development of these.

It is unquestionably true that during favorable seasons of the year, there is developed in certain of our soils a rich growth of algae. This is probably confined to the surface layers. To what depth algae extend will depend largely upon the texture of the soil, its ventilation and methods of cultivation. It is probably true, however, that the top crust of soil, the first inch or less, is usually too dry to favor algae. Irrigation may play a part in determining the distribution of soil algae. Whether or not our unirrigated soils possess an algal flora remains to be found out. But it can be readily understood how the turning of water on to an unirrigated area would introduce from the streams an abundance of algae. Although evidence is still insufficient, it is within the bounds of reason to believe, from these preliminary investigations, that all of our ordinary cultivated soils, especially those under irrigation, are far richer in algae than is usually supposed to be the case. More than this, we venture to assert that soil algae play a far more important role in soil fertility than is generally believed. Unquestionably, the organic matter furnished by soil algae must be reckoned with as an important source of energy for the nitrogen fixing organisms.

### SUMMARY.

Algae occur abundantly in many cultivated soils of Colorado.

Twenty-one different species of algae were found in the soils examined.

With but two exceptions, all the species found belong to the blue-green algae (Cyanophyceae.)

The family Nostocaceae is best represented.

There is a predominance of forms possessing thick, gelatinous sheaths.

The algae occur in a variety of soil types, for example, sandy loam, clay loam, heavy clay, hard, gravelly clay, heavy adobe and river bottom silt.

The most prevalent species of algae are *Phormidium tenue*, *Nostoc spp.*, *Anabaena sp.*, *Nodularia harveyana* and *Stigonema sp.*

In many Colorado soils, algae may be considered as an important source of energy for *Azotobacter*.

## EXPLANATION OF PLATES.

### PLATE I.

- Fig. 1. *Oscillatoria formosa*.
- Fig. 2. *Phormidium inundatum*.
- Fig. 3. *Phormidium subuliforme*.
- Fig. 4. *Phormidium tenue*.
- Fig. 5. *Phormidium valderianum*.
- Fig. 6. *Microcoleus vaginatus*, filaments within sheath.
- Fig. 7. *Microcoleus vaginatus*, single filament.
- Fig. 8. *Nostoc* "A", small form
- Fig. 9. *Nodularia armorica*.
- Fig. 10. *Nodularia armorica*, gonidia.
- Fig. 11. *Nodularia harveyana*.
- Fig. 12. *Nodularia harveyana*, gonidia.
- Fig. 13. *Nostoc commune*.

### PLATE II.

- Fig. 1. *Stigonema* "A", gonidia (?).
- Figs. 2, 3, 4. *Stigonema* sp
- Figs. 5, 6, 7. *Rivularia* "A".
- Figs. 8, 9, 10. *Rivularia* "A", young forms.
- Figs. 11, 12. *Rivularia* "B", young forms.
- Figs. 13, 14. *Pleurococcus vulgaris*.
- Fig. 15. Unicellular green alga, resting form, the contents dividing up into motile bodies.
- Fig. 16. Unicellular green alga, resting stage.
- Fig. 17. Unicellular green alga, motile body.
- Fig. 18. *Navicula*, a diatom.

### PLATE III.

- Figs. 1, 2. *Stigonema* "A".
- Fig. 3. *Nostoc* "A", vegetative filament.
- Fig. 4. *Nostoc* "A", gonidia.
- Fig. 5. *Anabaena* "A", vegetative filament.
- Fig. 6. *Anabaena* "A", filament producing gonidia.
- Figs. 7, 8, 9. *Anabaena* "A", germinating gonidia.
- Fig. 10. *Nostoc* "C", vegetative filament.
- Fig. 11. *Nostoc* "C", gonidia.
- Figs. 12, 13, 14, 15, 16, 17, 18. *Stigonema* sp., developmental forms.

### PLATE IV.

- Fig. 1. Soil capillarity tube a 10-inch section from the 7th to 17th inch.
- Fig. 2. *Nodularia* "A", filament producing gonidia.
- Fig. 3. *Nodularia* "A", vegetative filament.
- Figs. 4, 5. *Nodularia* "A", germinating gonidia.
- Fig. 6. *Nostoc* "B", gonidia.
- Fig. 7. *Nostoc* "B", germinating gonidium.
- Fig. 8. *Nostoc* "B", vegetative cells and gonidia.
- Fig. 9. *Nostoc* "B", vegetative cells and gonidia.
- Fig. 10. Flask culture of soil sample No. 1.