

The Agricultural Experiment Station

OF THE

Colorado Agricultural College

THE NITRIFYING EFFICIENCY OF CERTAIN COLORADO SOILS

BY

WALTER G. SACKETT

The Agricultural Experiment Station

FORT COLLINS, COLORADO

THE STATE BOARD OF AGRICULTURE.

	Term Expires
HON. F. E. BROOKS.....Colorado Springs,	1915
HON. J. S. CALKINS.....Westminster,	1915
HON. J. C. BELL.....Montrose,	1917
HON. WILLIAM HARRISON.....Clifton,	1917
HON. CHAS. PEARSON.....Durango,	1919
HON. R. W. CORWIN.....Pueblo,	1919
MRS. J. B. BELFORD.....Denver,	1921
HON. A. A. EDWARDS.....Fort Collins,	1921
GOVERNOR E. M. AMMONS, {	Ex-Officio
PRESIDENT CHAS. A. LORY, }	
L. M. TAYLOR, Secretary	
T. S. JONES, Treasurer	

EXECUTIVE COMMITTEE IN CHARGE

A. A. EDWARDS, Chairman

J. S. CALKINS

E. M. AMMONS

STATION STAFF

C. P. GILLETTE, M. S., Director.....	Entomologist
W. P. HEADDEN, A. M., Ph. D.	Chemist
G. H. GLOVER, M. S., D. V. M.	Veterinarian
W. G. SACKETT, B. S.	Bacteriologist
ALVIN KEYSER, A. M.	Agronomist
J. O. WILLIAMS, B. S. A., U. S. Expert-in-Charge	Horse Breeding
E. P. SANDSTEN, Ph. D.	Horticulturist
B. O. LONGYEAR, B. S.	Botanist
G. E. MORTON, M. L., B. S. A.	Animal Husbandry
E. B. HOUSE, B. S. (E. E.), M. S.	Irrigation Engineering
V. M. CONE, B. S.	Irrigation Investigations
R. E. TRIMBLE, B. S.	Assistant Irrigation Investigations
P. K. BLINN, B. S., Rocky Ford.....	Alfalfa Investigations
EARL DOUGLASS, M. S.	Assistant Chemist
S. ARTHUR JOHNSON, M. S.	Assistant Entomologist
L. C. BRAGG	Assistant in Entomology
J. W. ADAMS, B. S., Cheyenne Wells	Agronomy Assistant, Dry Farming
ELWOOD D. ROOD, A. B.	Assistant Chemist
W. W. ROBBINS, M. A.	Assistant Botanist
PAUL S. JONES, B. S.	Assistant Irrigation Investigations
RALPH L. PARSHALL, B. S.	Assistant Irrigation Investigations
JAMES D. BELL, B. S.	Assistant Irrigation Investigations
I. E. NEWSOM, B. S. D. V. S.	Veterinary Pathologist

OFFICERS

CHAS. A. LORY, LL. D.	President
C. P. GILLETTE, M. S.	Director
L. M. TAYLOR	Secretary
MARGARET MURRAY	Executive Clerk

THE NITRIFYING EFFICIENCY OF CERTAIN COLORADO SOILS.

BY WALTER G. SACKETT

INTRODUCTION

When our bacteriological studies of Colorado soils were begun some three years ago, the chief interest in the investigation centered about the occurrence and origin of certain "brown spots" in the soil. These had been observed as early as 1892, and by this time, 1910, they were becoming so common as to be identified readily by mere mention of their physical characters.

One not familiar with their appearance might easily mistake the smaller areas for spots where crude oil had been spilled, especially if this material had been used for heating purposes in the orchard where they occurred. Sometimes the spots are sharp in outline and are limited to a space of three to five feet in diameter; again, tracts of one to five acres and more exist, which present an unbroken expanse of dark brown, wet-appearing waste land. A well defined crust is usually present, and while the surface appears wet to the eye, it is not necessarily so; in fact, we often find it to be hard and dry. Beneath this crust, which may be one-half an inch in thickness, the soil is frequently mealy in texture. The same characteristic greasy, brownish-black color exists along the ditch banks, on the sides of the irrigating furrows and for miles in more or less broken stretches along the road-sides.

The first complaint of serious damage to agriculture from this cause was received by Dr. Headden in 1905, who, two years later, began his studies of the niter soils of Colorado. The universal grievance of those who are attempting to cultivate land of this character is that nothing will grow on the brown areas; it matters not whether the crop is sugar-beets, alfalfa, small grains, or fruit, all suffer alike. In looking over a field of oats or sugar-beets, one cannot fail to be impressed with the dark green color and rank growth of the plants which border the barren areas. This would seem to indicate the presence of a zone where conditions for plant growth are particularly favorable, beyond which they become almost intolerable. That such is the case has been demonstrated repeatedly by Dr. Headden. It suggests that where the growth is vigorous, the soil contains the optimum amount of nitrates for vegetative development; and where nothing can live, that the nitrates are in excess. Apple trees appear to have suffered more than any other of our fruit trees. In the fruit-growing sections of the state, these have died by the thousands, and acres of once valuable and profitable land are now worthless. Heavy manuring, additional draining, excessive irrigation and more thorough cultivation, all have been tried with the hope of ameliorating the pending conditions. Little or no benefit has accrued from these efforts, and up to the present time, we have observed nothing which seems to indicate a positive recovery, although, now and then, our attention has been called to cases where the intensity of the attack, as measured by the time required to accomplish

the destruction of the crop, appears to have been lessened somewhat.

A full description of the characteristic appearance and manner in which fruit trees and cultivated crops succumb to the trouble, here referred to, has been given elsewhere (1) and a further elaboration of this feature is unnecessary at this time.

This condition of the soil which has resulted so seriously to the agricultural and horticultural interests of the state has been shown by Dr. Headden to be due to the presence of large quantities of nitrates. To one who is accustomed to think of these salts in thousandths and ten-thousandths of a per cent., as they occur in the east and south, the quantities which we find in our niter sections must seem preposterous. However, one visit to the troubled districts is usually sufficient to convince the most skeptical. It is not uncommon to find the nitrates in some of the brown spots making up from two to six per cent. of the weight of the air dried soil. From this, it will be seen at a glance that our problem is not one involving pounds but rather tons of nitrates. For example, we have the analysis of the soil from an orchard where the samples were taken to a depth of one foot; it carried 2.873 per cent. of nitrates, corresponding to 113,480 pounds or 56.74 tons per acre foot. This does not represent an isolated brown spot a few feet in diameter but an area of ten to twelve acres, being a portion of a forty acre tract; again, in another sample, taken to a depth of five inches, the area involved being about eight acres, sodic nitrate corresponding to 344,000 pounds or 172 tons was found; in the top five inches of another eight acre tract the equivalent of 189,971 pounds or 95 tons was present. Dr. Headden has examined sample after sample from the affected districts and all his analysis tell essentially the same story—excessive nitrates.

The difference which we find between the niter content of the brown spots and that of the adjoining apparently normal soil is rather striking. A sample of the brown crust from the side of an irrigating furrow in an apple orchard contained 640 parts per million of nitrate nitrogen, while two and one-half feet away, the surface four-inches of normal soil gave 34 parts. The surface half-inch from the top of the ridge of an irrigating furrow in a deserted oat field including the brown crust showed 1360 parts per million of nitrate nitrogen, while six feet from the edge of the mottled brown area, the normal soil contained but 46 parts. To the writer, this seems to suggest rather strongly the probability that the brown patches are purely local surface-formations, which are actively engaged in building up nitrates *in situ*, especially since the areas are increasing daily both in number and in size. Furthermore, we are now in position to present a complete chain of experimental evidence to show beyond all reasonable doubt that these niter areas in the incipient stage of their formation, if not in the later stages as well, contain within themselves all the agents necessary to bring about their existence.

(1) Bakteriologische Untersuchungen über die Stickstoffbindung in gewissen Bodenarten von Colorado. Cent. f. Bakt., II Abt., Bd. 34, No. 4-7. S. 81-114, 1912.
Bacteriological Studies of the Fixation of Nitrogen in Certain Colorado Soils, Bul. 179, Colorado Experiment Station, 1911.

There is no need of attempting to trace the origin of these accumulations to the concentration at the surface of the salts in the ground waters containing a hypothetical nitrate; moreover, granted that a ground water could be found independent of a niter area, which was rich in nitrates and which underlay the tract in question, why should we not find the nitrate and the brown color, as well as the sulfates and chlorides, distributed uniformly over the hundreds of square miles which are involved in this trouble? This is not the case.

In this connection, it may be said that Dr. Headden had made a very careful study of the ground-waters of Colorado previous to the appearance of the niter trouble and had failed to find more than a mere trace of nitrates. Since then, he has followed up this phase of the question, and except where the ground-water flows under a niter area, from which it unquestionably receives the salt by leaching from above, he finds the nitrates present only in negligible quantities. In order to obtain the quantity of nitrates from our ground-waters which we meet in the brown spots, the evaporation of many acre feet of water and many years of time would be required. Furthermore, the chlorides and sulphates which would come from the evaporation of this immense amount of ground-water would be sufficient to cover the land with a white deposit many feet deep. Illustrative of the case, Dr. Headden (1.) gives the following:

"We will not consider any greater depth of soil than is represented by our samples, i. e., two inches, but we will calculate how much of this drain water would be required to furnish the nitrate which we actually find in these two inches of soil, and we take this as 2,150 pounds. The samples of soil and drain water were taken in May, 1911. The drain water contains 0.1 part per million of nitric nitrogen, equivalent to 0.6 parts per million sodic nitrate; taking an acre foot at 2.7 million pounds it gives us 1.62 pounds of nitrates per acre foot of water and we would have to evaporate 1,327 acre-feet of this drain water to obtain this 2,150 pounds of nitrates which we find present at this time. The evaporation of this amount of water would require, assuming that the annual evaporation amounted to sixty inches (At Fort Collins it is only 41 inches) two hundred and sixty-five years. This drain water carries 8,489 parts of total solids per million which, calculated on the 1,327 acre-feet of water necessary to yield the 2,150 pounds of nitrates, would yield 30,414,840 pounds of salts, a quantity sufficient to cover the land more than seven feet deep, if we suppose them to have the same density as the soil itself."

"The changes in the conditions of these soils have taken place within the past few, say six, years and these conclusions to which we are forced, if we suppose that these nitrates have their origin in the ground-waters, are evidently false. We know that no 1,327 acre-feet of water have evaporated to dryness on this land in this time and it is evident that our country is not covered nearly eight feet deep with

(1) Fixation of Nitrogen in Some Colorado Soils, Wm. P. Headden, Bul. 178, Colo. Exp. Sta., p. 28, 43, 1912.

calcic chlorid and other salts and we are likewise quite as sure that land which up to six years ago was good, and this assumed period is from three to six times as long as the facts indicate, has not been two hundred and sixty-five years in going to the bad."

That these spots are the remains of great herds of extinct animals which perished from some unknown cause is highly improbable: First, because the areas involved are too great; second, as mentioned before, the present spots are increasing in size; and, third, spots are appearing today in localities where the trouble has never been reported before.

For the same reasons, there is no ground for believing that these areas are niter beds related to any established geological horizon.

Having failed to find a satisfactory explanation for these brown areas in any of the foregoing hypotheses, we ventured the assumption that the nitrates were and are being formed *in situ* and proceeded with the hypothesis that they owe their origin to the activity of microorganisms.

To assume that ammonifying and nitrifying bacteria alone were responsible for the formation of the nitrates was to assume the existence of large quantities of organic matter in the soil, which, as a matter of fact, is quite deficient. This condition obtaining, we were obliged to begin our investigation one step farther back and to find some adequate supply of organic nitrogen which could furnish protein for the ammonifying organisms.

FIXATION OF NITROGEN

In Bulletin 179, we have shown that our niter soils are abundantly stocked with *Azotobacter chroococcum* by virtue of which they are able to build up large quantities of proteid nitrogen from atmospheric nitrogen. Not only have we demonstrated the almost universal occurrence of the nitrogen fixing organisms, but we have also been able to obtain a positive increase in the total nitrogen content of the soil itself by merely keeping the soil moistened and holding it at 28 degrees C. Without any inoculation other than that which the soil already contained and without the addition of any carbohydrate to supply energy, we were able to secure in thirty days an increase in the total nitrogen of 11.12 per cent. with one sample and 9.83 per cent. with another.

So far as a supply of carbon in the soil with which to furnish *Azotobacter* with the necessary energy for nitrogen fixation is concerned, I may say that Professor Robbins (1) has reported to date the occurrence of some twenty-four species of algae. All but two of these belong to the blue-green algae (Cyanophyceae), the family Nostocaceae being best represented.

BROWN COLOR

The brown color of the niter soils has been discussed quite fully in

(1) Algae in Some Colorado Soils, W. W. Robbins, Bul. 184, Part II, Colo. Exp. Sta., 1912.

Bulletin 179 so that mere reference to it will suffice at this time. In the light of our experiments, we have little hesitancy in saying that this is due in a large measure to the pigment of *Azotobacter chroococcum*. Even the colorless varieties in the presence of .05 per cent. of sodium nitrate produce a dark chocolate brown pigment. This experiment has been carried out repeatedly on the ordinary solid media, ground quartz, sand and an assortment of soils. Given a source of energy, the nitrate appears to be the limiting factor in the formation of the brown color by the nitrogen fixing organisms.

AMMONIFICATION

In Part I of Bulletin 184, we have taken up a study of the ammonifying efficiency of this same class of soils, and we have been able to show that so far as the presence of an active, ammonifying microbial flora, as well as suitable medium for their development for converting the protein of the *azotobacter* cells into ammonia, our soils are all that could be desired. The results of our experiments, based upon the change of the protein of alfalfa-meal, cotton-seed-meal, flaxseed-meal, and dried blood into ammonia led us to believe that this is a property common to many cultivated Colorado soils; that soils in the incipient stage of niter trouble surpass our normal soils in ammonifying efficiency; also that compared with the soils from other localities our niter soils excel in this function to a very marked degree. The maximum per cent. of ammonia produced in seven days by any soil from 100 m. g. of nitrogen as cotton-seed-meal was 51.98 per cent.; as dried blood 52.64 per cent.; as alfalfa-meal 34.85 per cent.; as flaxseed-meal 12.15 per cent.

NITRIFICATION

Having shown that the niter soils could transform atmospheric nitrogen into ammonia salts by the two processes just outlined, we had yet to demonstrate their ability to change the ammonia compounds into nitrates before we could assert definitely that the atmosphere is in all probability contributing the nitrogen for the excessive nitrates. In the pages which follow, will be found a detailed discussion of our studies upon the nitrifying efficiency of these soils,—nitrification being the third and final step in the transformation of the atmospheric nitrogen into nitrates. I use the term *nitrifying efficiency*, rather than *nitrifying power*, in the sense in which it has been employed by Stevens and Withers (1) to denote not only the presence of the nitrifying organisms in the soil which are capable of exercising their specific function under favorable conditions (nitrifying power) but also the suitability of the soil as a medium in which the process of nitrification may proceed advantageously (nitrifying capacity).

SOIL SAMPLES

We have not confined the investigation to Colorado soils alone.

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

but have extended it to include samples from widely separated and distant localities in the United States. These have been designated as "foreign" merely to distinguish them from the local collection. With but two or three exceptions, as hereinafter noted, they have been gathered from cultivated land and represent cotton, tobacco, melon and orchard soils of the South, general farming and sugar-beet land of the East, Central and Middle West, the citrus and viniferous districts of the Southern Pacific Coast, and the orchard section of the Northwest.

Their distribution was as follows: Two samples from Georgia, three from North Carolina, one from Virginia, one from Ohio, one from Arkansas, one from Kansas, two from Oklahoma, one from Texas, nine from California, and one from Washington.

The samples from our own state have been taken from different localities over a continuous area of approximately six hundred square miles. Much of this is still good farm and orchard land but no small portion has been rendered worthless during the past five years by excessive nitrates. The soils which we have selected are fair representatives of orchard land, sugar beet, oat and alfalfa fields, barren wastes and raw land in the afflicted district. A few samples from apparently normal localities have been introduced for comparison.

METHODS

Nitrifiable Substances Employed:

Ammoniacal nitrogen for our experiments was supplied to the soils in three forms: Ammonium sulphate, ammonium carbonate and ammonium chlorid. An additional series was prepared with dried blood in order to determine whether the ammoniacal nitrogen made from proteid nitrogen by the ammonifying bacteria would respond to the nitrifying agents to a greater or less degree than the above mentioned compounds of ammonium. The ammonium sulphate, ammonium chlorid and ammonium carbonate were added to the soils in the form of solutions so prepared that 10 c. cm. of each solution contained its respective salt in quantity sufficient to furnish 100 m. g. of nitrogen.

Collection of Samples.—With respect to the collection of the foreign samples, it was practically impossible either to expect or to secure any uniformity since the majority of the samples were taken by persons wholly unfamiliar with the technique of sampling, to say nothing of bacteriological precautions. To have imposed upon them the burden of sterilizing spatulas and containers would have meant not getting the soils; consequently, the only instructions which accompanied the requests were that the samples be taken to a depth of four inches and that about as much soil be sent as would go into an ordinary sized cigar box. The majority of collectors lined the boxes with paper before putting in the soil, but some failed to do even this. Occasionally, a sample came to the laboratory in a heavy, paper sugar sack, and one was received in a cloth bag.

For the benefit of any who may take exception to the results of our comparative studies on the ground that the foreign series

was not collected according to the usual methods employed by soil bacteriologists, I may say that no one recognizes the value of using standard methods, wherever possible, more than we do, and we have the greatest respect for the investigator who holds firmly to this position. However, for our purpose, I believe that our soils were just as nearly representative of their respective localities as if they had been taken by an experienced person under aseptic conditions.

In Colorado where we could give our personal supervision to collecting the samples, we have exercised every precaution to prevent exterior contamination. All of the surface litter was removed before opening up the ground. Except where otherwise stated in the descriptions which follow, the samples were taken to a depth of six inches, i. e., they included the surface six inches; the soil was removed with sterilized spatulas and placed immediately in double sterilized, paper sugar sacks; each sample contained approximately five pounds of the moist soil. All of the samples were shipped by express, as soon after collection as possible, to the bacteriological laboratory of the Experiment Station at Fort Collins in order to minimize the time in transit, during which, 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. As a result of our nitrogen fixing and ammonifying studies, we have made it a point in collecting samples where the brown color is present, to avoid getting this portion of the soil since experience has taught us that the nitrates are apt to be so high that they interfere with the normal development of the microorganisms present.

Preparation of Samples.—From this point, both the Colorado and the foreign soils were handled alike. As soon as they reached the laboratory, each was emptied on a sheet of heavy, sterilized manilla paper and thoroughly mixed. It was next divided into two unequal portions, the larger being spread out in the air to dry in diffused light, while the smaller was transferred in its original moist condition to a sterilized Mason fruit jar and sealed. As soon as air dry, which seldom requires more than twenty-four hours in our atmosphere, each soil was again mixed and pulverized in a glass mortar. Both the mortar and pestle were sterilized carefully between each two different samples with a 5 per cent. solution of lysol. They were subsequently rinsed with hot, boiled water and allowed to dry before the next sample was treated. The soils were next passed through a thirty mesh wire sieve which was washed between the different samples and sterilized by dry heat.

As containers for the prepared soil, we have used the same style of deep culture dish as in our previous experiments. These are similar in shape to the ordinary Petri dish except that they are deeper, measuring 10 cm. in diameter and 4 cm. in depth.

100 grams of soil, prepared as described above, were used for each dish. The ammonium sulphate, ammonium chlorid and ammonium car-

bonate were mixed with this by adding the respective solutions drop by drop to a portion of the 100 grams, and then more of the soil was added and more solution, and so on until 10 c. cm. of solution corresponding to 100 m. g. of nitrogen had been used with the whole sample. For the organic nitrogen, dried blood (1) corresponding to 100 m. g. total nitrogen was added to like portions of soil and was thoroughly incorporated by stirring the mixture for five minutes with a sterile glass rod. Five cultures were prepared from each of the Colorado soils. Three received the ammonia salts, one the dried blood, and one to which no form of nitrogen was added, was kept as a control. The ammonium chlorid series was omitted from the foreign soils.

Inoculation of Soils.—Each culture mixture was inoculated with 10 c. cm. of soil infusion prepared from fresh soil of the same source as that already in the dish and corresponded to 5 grams of the undried material. These infusions were made by shaking 100 g. of undried soil with 200 c. cm. of sterile distilled water for five minutes. They were allowed to stand thirty minutes for the coarser particles to settle after which 10 c. cm. of the turbid suspension were drawn off with a sterile pipette and distributed uniformly over the surface of the soil in the culture dish. In order that the dried blood and control series might have the same moisture content as the other three, sufficient sterile distilled water was added to make the moisture approximately 22 per cent. Additional water was used for the dried blood at the rate of 1.5 c. cm. for each gram of material.

The weight of each culture was determined at the beginning of the experiment and the loss of water by evaporation was restored every ten days with sterile, distilled water.

Incubation of Samples.—All of the cultures were kept in the incubator at a temperature of 28-30 degrees C. throughout the experimental period of six weeks. At the end of this time quantitative determinations were made for nitrites and nitrates.

Chemical Methods.—10 grams of each sample, air dried and pulverized, were used in the chemical determinations. This was extracted with hot water and washed free from chlorides and nitrates on a Buchner funnel containing asbestos. The resulting extracts were rendered colorless by means of carbon black. That portion of the extract which was used for the determination of nitrites and nitrates was freed from chlorides by the use of silver sulphate. The loss of nitrous and nitric acid during the evaporation and concentration of the extracts on the water bath was prevented by the addition of milk of lime in excess.

The nitrates were determined by the phenoldisulfonic acid method; the nitrites by Ilsoyay's modification of Griess's test (sulfanilic acid and naphthylamine acetate); the chlorids, volumetrically, by titration with silver nitrate.

(1) The dried blood contained 13.0503 per cent. total nitrogen.

DESCRIPTION OF THE SOILS

COLORADO SOILS

Sample No. 75.—The first soil that we shall consider was collected at Fort Collins April 29, 1913. The land had been in barley the preceding year and in sugar-beets the year before that. It is what would be considered a normal clay soil for this section of the state; level, well drained, and free from any trace of white alkali and brown discoloration. In 1913, it produced an average of 45 bu. of wheat to the acre and in 1911, 24 tons of sugar-beets per acre. This sample was composed of twenty sub-samples taken to a depth of six inches over an area of one-half acre. The nitric nitrogen amounted to 7 p. p. m. and the chlorin to 500 p. p. m.

Sample No. 76.—For the next sample, we went to an altitude of almost 9000 feet to a truck patch nestled in a gulch on the outskirts of a mining town. This is the only tract of its kind which Nature has allowed in the vicinity and which, I should judge, has an area of less than twenty acres. It is more than 3000 feet above and 36 miles from any known niter area, and, so far as could be learned, it is perfectly normal. However, it has an added interest from the fact that previous to its present ownership it was held as a placer gold claim. The soil is a deep river-bottom silt loam and has been maintained in a very productive condition in recent years by the heavy application of stable manure. In 1911, the owner harvested 240 sacks (over 450 bushels) of potatoes to the acre. All kinds of vegetables and small fruits, especially strawberries, are grown here very successfully. At the time this sample was collected, May 11, 1913, nothing had been planted, and the ground was covered with a very liberal dressing of manure. This was removed as far as possible before taking the sample, but the heavy snows of the preceding winter and spring must have caused some leaching. Just how much this had affected the nitrates of the surface-layers, we have no means of knowing, but it undoubtedly contributed something. The nitric nitrogen amounted to 54 p. p. m. and the chlorin to 400 p. p. m.

Sample No. 77.—This soil came from a tract that we have had under observation since 1908. At that time it was planted to oats, but the stand was very spotted. Throughout the field barren patches were developing, brown in color and mealy in texture. Alfalfa growing had been abandoned in previous years because it was believed that the water table was getting so close to the surface that this crop could not succeed. In the light of our present knowledge, it is possible that it was the excessive nitrates rather than too much water that was injuring the alfalfa. In 1909 sugar-beets were planted, but with no better results. The brown areas kept increasing in number and size until there were patches of half an acre in extent where nothing would grow. That fall it was planted to winter wheat, but before the harvest of 1910, the whole twenty-five acres had died. I visited the place in 1911 and found that Russian thistles and salt bush had taken

possession of practically the entire area. Evidently the nitrates had been reduced by the heavy rains of the preceding winter and spring to a point of tolerance for these weeds since in other years nothing of this kind had occurred. I do not know what crop, if any, occupied the land in 1912, but when the present sample was collected in May, 1913, it had been sown to oats. The stand was exceedingly thin and the bulk of the growth was confined to the narrow zone adjacent to the irrigating furrows. Between these, the plants were very scattering. There was considerable white alkali in evidence, and the sides of the irrigating furrows bore a slight crust beneath which the soil was mealy. There were no well defined brown or chocolate colored areas as in previous years, but the whole field presented a marked brownish aspect. The soil is a clay loam and contained nitric nitrogen at the rate of 26 p. p. m. and chlorin 4,500 p. p. m.

Sample No. 78.—While our chief interest in this investigation attached itself to cultivated soils, we have selected, nevertheless, one sample from the side of an adobe hill where the soil was just being formed from the weathering shales. This was raw land, of course, if it could be styled land at all, and there was no evidence that anything had ever grown upon it. The overlying mesa was in cultivation, and judging from the water erosion of the hillside, it had received some of the leachings from above during heavy rains. Such formations as this, by gradual weathering, are contributing quite extensively to the formation of our heavy adobe clay soils and it was of considerable interest to learn whether the ancestors of these soils were as active biologically as their cultivated offspring. There was no white alkali visible on the surface and no seep from the side of the hill at the time the sample was taken, May 13, 1913. The nitric nitrogen was higher than I should have expected had it not been for the wash from above; it amounted to 140 p. p. m. and the chlorin to 700 p. p. m.

Sample No. 79.—The next sample came from a well drained orchard where there was never any white alkali to be seen, but where the niter trouble has been manifesting itself to a greater or less degree for the past four years. When it first made its appearance it was confined to a few rows of trees on the west side of the orchard; today, it extends up and down both sides of the main highway for several miles, easily recognized by the dark brown, greasy appearance of the soil. That section of the orchard from which this sample was taken is a red clay loam, and owing to this color it has always been difficult to discern any decided brown discoloration, although in another part where the formation is different, the brown color is strongly developed.

In 1910, the burning was very moderate and there seemed little reason for anticipating any grave danger from this cause. A few trees had died, but most of those affected appeared to be dying slowly rather than to be going in one season as is often the case. The next year, the number involved was considerably greater and the burning continued to be relatively mild, but by 1913, although the disaster had been moving slowly, it had been accomplishing its work just as surely,

and nearly every third tree in the twenty acres had been killed and removed. When I visited here in May, 1913, young apple trees had been set out where the old trees were missing. I have not been back since to see if they survived the summer, but I should be surprised if they did. Red clover had been sown between the trees as a shade crop, but the stand was very scattering.

My sample was collected about two feet from a hole where a dead tree, presumably killed by niter, had been taken out. When the nitric nitrogen was determined, I was very much surprised to find that it contained but 5 p. p. m. with 1000 p. p. m. of chlorin. That the nitrates in this soil had been considerably above normal in 1910, there is no question, for at that time Dr. Headden found in the surface-three inches 4.902 per cent. of water soluble salts to be nitrates, the water soluble amounting to 2.92 per cent. of the air-dried soil. I am at a loss to explain the low figure that I obtained unless it is that I was misled by the hole where the tree was missing into thinking that it was a niter tree when, in reality, it had died from another cause, or else, I just happened to take the sample from a spot where the excessive nitrates had not yet developed. As mentioned before, there was no brown color apparent on this red soil, to guide one either in picking out or in avoiding the bad places.

Sample No. 80.—In our study of this question we are continually meeting land-owners who believe that the difficulty can be remedied by proper drainage. The present case is only one of several that could be cited to show that little relief follows the installation of a drainage system. It is difficult to see how it could be otherwise, since the accumulation of nitrates does not appear to be a question of drainage; again, it has been found to be practically impossible to drain the heavier niter soils because of their sticky, gumbo character; and furthermore, in the majority of instances, draining is not indicated and is unnecessary. However, in the case at hand, the owner employed an experienced engineer to put in the necessary amount of tile at the proper depth and with the correct fall and distribution.

A part of the orchard was manifestly seeped as indicated by the white alkali on the surface, and this condition was remedied considerably by the drain, but so far as checking the progress of the niter destruction, nothing had been accomplished.

When I collected my sample, on the thirteenth of May, 1913, about one-third of the trees were dead and the barren spots had been reset with young stock. The soil is a clay loam and where the sample was taken the surface was very fine and mealy. Because of this condition, the surface-inch was removed and the second to fifth inch used. The nitric nitrogen present amounted to 13 p. p. m. and the chlorin to 10,400 p. p. m. As in the preceding orchard, we have observed here also in previous years that the burning was moderate and that the trees were succumbing slowly. This was probably due to the fact that the nitrates were accumulating more gradually than in other localities where damage had been more acute.

Sample No. 81.—This soil came from a field which had been

in cultivation at one time, but which has been abandoned for the past two years, at least, for some unknown reason. The tract is situated well above the surrounding country on an imposing mesa, but in spite of its favorable location and elevation, it is a matter of common observation that much of the land is too wet for successful farming without a great deal of draining. Why this condition should exist where every advantage appears to be offered for natural drainage has been some what of a quandary. During recent years, very extensive ditches and drains have been put through this section and incidentally we observed while the trenches were still open that the underlying shales present at their upper limit a series of basins, filled with coarse gravel, which appear to retain the irrigating waters and in this way interfere with natural drainage.

The field in question was barren for the most part, although clumps of Russian thistles occurred here and there. The ground was wet in places from an over-flowing ditch and there was a very decided development of large brown areas. Everything indicated that these were niter spots. Accordingly, I took a composite sample of the brown surface-inch from a number of dark brown patches on the ridge of what looked to be an old sugar-beet row.

The soil is a clay and the surface color varied from a yellowish brown to chocolate. A determination of the nitric nitrogen showed it to contain 1360 p. p. m. with 32,400 p. p. m. of chlorin.

Sample No. 82.—This sample was taken exactly six feet from the brown area described in No. 81 and consisted of the second to fifth inches. The soil was very dry and rather mealy on the surface, but there was no indication whatever of any brown color. Russian thistles had grown on this particular spot luxuriantly and had to be removed before securing the sample. This soil, from just beyond the limits of the brown areas, together with No. 81 from the brown crust gave me an excellent opportunity to compare the microbic activities under two such different soil conditions where the two samples occurred less than seven feet from one another. The soil contained 46 p. p. m. of nitric nitrogen as compared with 1,360 in the brown crust. The chlorin present amounted to 9,800 p. p. m.

Sample No. 83.—This soil came from an orchard in prime bearing condition where seven trees had been killed by niter in 1911. These trees were all in one row, although not adjacent. They were cut down in the fall of 1911 and when I visited the orchard in September, 1912, the water sprouts from these stumps showed very severe nitre burning. My sample was taken between two of the stumps and consisted of the surface-three inches. The soil is a very heavy clay. No new cases of burning had been observed in the orchard that summer and there was no indication of the trouble spreading to adjacent rows. Seven of the trees in another row bore rather scant foliage and small fruit but this condition might have been due to a variety of causes. This soil contained 6 p. p. m. of nitric nitrogen and 58 p. p. m. of chlorin. I should consider this orchard in the incipient stage of the trouble.

Sample No. 84.—Another bearing orchard, probably fifteen to eighteen years old, in the same neighborhood as No. 83 gave us the next sample. The soil here is a heavy clay. When I visited the ranch in September, 1912, fifteen trees, all within a circular area in one corner of the orchard, were burning very badly. The foliage was practically all brown but the apples showed no signs of any injury and unless some very rapid change for the worse took place, they gave promise of maturing. In a remote corner of the orchard, three more trees were found to be buring. The soil between the trees showed no brown spots as yet, but the sides of the main ditch were brown four or five feet up from the water. My sample was taken near a burning tree and contained only a trace of nitric nitrogen and 128 p. p. m. of chlorin. Here also this orchard is to be looked upon as being in the incipient stage of the trouble.

Sample No. 85.—We have here another old orchard where the burning from excessive nitrates has been present to our knowledge for four years. In 1910, the attack was very acute and about two and one-half acres of bearing trees were destroyed. Strangely enough, since then the onslaught has abated, and while a large number of the trees show the typical burning of the foliage, but few more have died. A considerable number are making practically no growth and are struggling along stunted and half leaved out. The soil is a clay loam and shortly after it is irrigated, the brown color can be seen very readily, developing along the irrigating furrows. The sample was taken in September, 1912, near an affected tree and contained 6 p. p. m. of nitric nitrogen and 1000 p. p. m. of chlorin. The soil was very dry and hard at that time and no brown color was in evidence near where the sample was collected, although it had been seen in great abundance on other occasions.

Sample No. 86.—Sample No. 86 is a sandy loam and came from the mealy ridge of an irrigating furrow in an orchard where high nitrates have made their appearance within the past three years. While collecting samples in this vicinity in former years, I had passed by this place many times but never until 1911 had I observed anything unusual about either the trees or the soil. The area of the orchard is about four acres, and in October, 1911, every tree on the two acres was seriously affected, in fact, they were as good as dead. When I saw the place again in September, 1912, all of these trees had been grubbed out, and the two acres had been planted to oats and alfalfa. It goes without saying that the experiment had been disappointing. At least three-fifths of the trees on the remaining two acres were burning. A well defined crust had formed on a portion of the ground, and this was dark chocolate brown to black in color, all the darker because of its moist condition due to the presence of quantities of deliquescent magnesium nitrate. sample contained 130 p. p. m. of nitric nitrogen and 6400 p. p. m. of chlorin.

Sample 87.—This soil came from an orchard which was among the first to be called to our attention because of the heavy losses from high

nitrates. The area of the original tract was forty acres and previous to 1911, from seven to eight acres had died and the trees had been taken out. In 1911, this part was sown to oats, but the stand was very unsatisfactory. The mealy ridges of the irrigating furrows were barren, and what little growth occurred took place in the bottom of the furrows where the water appeared to have reduced the nitrates to a point of partial tolerance. Nothing was planted here the next year. The burning was general over the remainder of the orchard in 1912, but the attack seemed to have been concentrated upon two rows of trees next to the outside row on the east side. Practically every one of these was so badly burned that no green color was left to the foliage and the apples in September were not more than two-thirds grown and badly shriveled. Judging from the hardness of the ground and the aspect of the orchard as a whole, it had been neither irrigated nor cultivated that season and was suffering from general neglect. My sample consisted of the surface-four inches taken between two burning trees. The soil is a clay loam and was very hard and dry. There was no brown color visible, due, presumably, to the fact that the ground was too dry for the development of the *Azotobacter* pigment. It contained 6 p. p. m. of nitric nitrogen and 202 p. p. m. of chlorin.

Sample No. 88.—Of all the soils which we have had under observation, none has offered a better opportunity for watching the development and lateral movement of the high nitrates than this one. The writer's acquaintance with the area dates from 1909 when all that was left of a twenty acre orchard was parts of six rows of trees on one side next to a ditch. The whole tract involved is forty acres, twenty of which was in alfalfa prior to 1907 and the balance in bearing apple trees. In 1907, barren spots began to appear in the alfalfa and brown patches to develop in the orchard. Soon the trees began to show the burned leaf margins which we have come to associate positively with excessive nitre, and a large number died. Here, as is so often the case, a few trees in the interior of the orchard succumbed first, and from these as a focal center, the destruction spread rapidly in all directions. The attack was so severe and the progress so rapid that by the spring of 1909, all of the alfalfa had been killed and at least fifty per cent. of the trees. During 1909, the remainder of the trees died except parts of six rows. During 1910, the three inside rows of these were killed. In 1911, the two inside rows of the remaining three travelled the road of their fellows, and the lone outside row bade fair to follow, for several of its members were burning. When I next visited the place to take the present sample in the fall of 1912, not one tree was standing. All had died save three, which the owner had taken pity on and cut down. These three were still green; the foliage was not burned, but the leaves were small and scattering. I first saw the orchard in 1909, and at that time the whole central portion from which the trees had been removed was as brown as could be; in fact, almost black. This color did not extend into the six rows which were still alive beyond the very inside row. The next year it moved in four rows, but beyond this, the color and physical condition of the soil were normal to all appearances. By

1912, when the last trees died, the brown color covered the entire area, spreading, in more or less broken stretches, over the ground occupied by the very outside tree-row. The soil in the beginning was a rather heavy clay loam; now it is quite mealy and ashy in character.

I do not know where we could find a better illustration of the formation and spread of nitrates from a central point than is given in this case by the regular succession in which row after row of trees went down before the approaching niter wave.

One rather interesting thing that occurred in connection with this soil was the very luxuriant growth of salt bush which took place in the summer of 1911. With the exception of a few barren spots, here and there, where the nitrates were evidently too concentrated, the whole forty acres were covered with this weed, waist high. During the preceding three years, nothing had grown there, but the winter and spring of 1911-1912, were very wet for this locality and it is possible that the nitrates were washed out sufficiently to allow the Atriplex to become established. While there were some weeds here in 1912, the vigor of the growth and the area covered were nothing compared with that of the preceding year. The tract was entirely barren in 1913.

My sample was taken between the two living trees that had just been cut, where the soil was beginning to get rather mealy. It consisted of the surface-three inches and contained 70 p. p. m. of nitric nitrogen and 8300 p. p. m. of chlorin.

Sample No. 89.—The sample was collected from an orchard where the trees have been dying since 1908. The owner believed that the cause of the trouble was starvation so he gave the soil a heavy dressing of stable manure, thereby, in all probability, aggravating the attack. At any rate, no benefit whatsoever resulted, and year after year more and more trees have succumbed until about three and one-half acres died and were grubbed out. One year the land was planted to spring wheat where the trees were removed, but practically none of it ever came through the ground. When I last saw the place in the fall of 1912, the burning had extended north and west into the good part of the orchard five more rows, but there was almost none to be found in the remaining five or six acres. The ground appeared to have been heavily manured quite recently. The soil was a coffee-brown color where the trees had been taken out, and no attempt was being made to use the land. It was not wet and there was no water in a test well at a depth of 6 feet, although spots of white alkali were showing up here and there. The soil is a sandy loam, not particularly mealy. The brown color had not yet developed, at least it was not visible at that time, where the trees were then burning and where I took my sample. Nitric nitrogen amounting to 180 p. p. m. and chlorin to 1600 p. p. m. were found.

Sample No. 90.—We have next the case of a ninety acre apple orchard where the trees commenced dying from too much nitrate in 1908. At this time the brown stain was most conspicuous on the sides of the irrigating furrows, but more recently it has spread over the entire surface in some parts of the orchard. During 1910-1911 the vio-

lence of the attack centered in one corner and resulted in the death of every tree on two and one-half acres save a few in the outside fence-row along an irrigating ditch. This meant the loss of approximately two hundred and fifty bearing trees. During the last two seasons, the trouble has become generally distributed over the south half, but it appears to be very much less active than in the preceding years. The trees exhibit some burning, and the leaves and fruit are undersized, with a suggestion of premature ripening, but the death rate does not begin to be as high as in 1910-1911. My sample consisted of the surface four inches of sandy loam taken between two burning trees on the edge of the two and one-half acres where everything had died. No brown color was in evidence, although the soil was moist. The nitric nitrogen present amounted to 13 p. p. m. and the chlorin to 140 p. p. m.

Sample No. 91.—During the summer of 1910, while looking over a large orchard for indications of niter, I came upon three trees near to one another whose leaves were considerably burned. The inference was that the same causal factors were operative here as elsewhere, although no nitrate determinations were made at the time. A sample of the soil was taken for my nitrogen fixation experiments, and better than 11.00 m. g. of nitrogen were fixed with 1.5 g. of mannite. I did not see the orchard again until the fall of 1912, and I must confess that I was very much surprised to be unable to find a single tree affected where I had noted them before. In place of the dwarfed, sickly growth with many brown leaves, I found the trees unusually vigorous and free from burning. Many had sent out new shoots over three feet long; the foliage was fully developed and of an excellent color. Confronted with such an unexpected condition of affairs, I set out at once to find the manager and to ascertain, if possible, an explanation of this remarkable rejuvenation. According to the owner's statements, one hundred and fifty-six trees were in a serious condition with niter symptoms in 1911, and realizing the immediate need of some extreme measures to save the trees, he plowed the whole orchard twelve to fourteen inches deep that fall, and in the spring of 1912, he dynamited the two acres where the trouble seemed to be localized using one-half stick of dynamite placed midway of the rows for each four trees. Following this, he turned on a big head of water and washed out the soil as thoroughly as can be accomplished by such a procedure. The blasting had loosened the soil to a considerable depth so that the water penetrated it very readily, and any nitrates that had accumulated in the surface layers were doubtless reduced very materially. Added to the benefit derived from this source was the greatly improved physical condition of the soil, which must have encouraged new root development. This last factor was undoubtedly responsible for the thriving condition of the orchard. Although I did not locate any burning trees myself, Mr.——— stated that there were a few, not to exceed six, whereas in 1911 there had been one hundred and fifty-six. He told me that the results of his blasting experiment had been so gratifying that it was his purpose to apply the same treatment to the remainder of the orchard before another season.

At no time have I observed any brown color on this soil. The present sample, a heavy clay, was taken from the two and a half acres described above and contained only a trace of nitric nitrogen and 00 p. p. m. of chlorin.

Sample No. 92.—This soil came from an orchard which is very favorably located for securing the best possible natural drainage. It borders on a river and is about twelve feet above the normal level of the stream. The soil is a light sandy loam and is underlaid at five to eight feet with coarse gravel. When the river is at flood, the level of the ground-water has been within ten inches of the surface and in the depressions the water has stood two inches deep so there can be no question about the openness of the soil. It has been frequently observed on this place that the ground water rises and falls regularly with any appreciable change in the level of the river.

The orchard is about twenty years old and until 1909 it had been very productive. During that year, the brown color became very conspicuous on the surface and the nitrates developed very rapidly. Approximately three hundred apple trees were killed, and in the spring of 1910, they were removed. The entire surface of four and one-half acres from which the trees had been taken was covered with a hard brown crust beneath which the soil was mealy and ashy. Corn was planted here, but it amounted to nothing since much of it never came up, and what little succeeded in getting through the ground turned yellow and died when it was 8 to 10 inches high. Not despairing of all hope, the owner planted it to cantaloupes in 1911. Here and there a plant got established and produced very well, but the crop as a whole was a dismal failure. The tract was sown to oats in 1912 but only those plants that were next to the water in the irrigating furrows survived. A good many got to be six to eight inches tall and then burned. In some places weeds were growing in the furrows, but in many others neither weeds nor oats could endure the nitrates and the stand was so poor that it was not considered worth irrigating. When I visited the ranch in the fall of 1912, the burning had extended no farther into the orchard than in 1911 and had stopped abruptly almost to the row. Strangely enough, there were a few scattering trees next to the river that were badly burned. One tree in particular attracted my attention, since it was not to exceed ten feet from the edge of the river bank and twelve feet above the water, yet it was as brown as could be from niter injury. Most certainly the burned condition of the leaves could not be attributed to poor drainage in this case.

My sample was collected from the barren area and contained 320 p. p. m. of nitric nitrogen with 7800 p. p. m. of chlorin.

Sample No. 93.—Thus far, all of the orchards which we have described have been apple, but we come now to a section of the country where peaches and apples are grown in alternate rows, the peaches being used as fillers. I first became interested in this orchard in 1910, not because of the niter but because of a peculiar physiological condition of some apricot trees. While looking about for some explanation of this trouble I noticed the same brown color on the sides of the irrigating

furrows. A short distance away I found about a dozen apple trees badly burned so that my diagnosis of the condition of the soil was confirmed.

In 1911 I visited the orchard and again in the fall of 1912, but on both occasions I failed to find either the brown color on the soil or burning of the apple foliage. The peach trees were yielding well and everything seemed to be normal. I took a sample of the soil, a heavy clay, however, as typical of our best peach land and have regarded it as a normal soil. It contained 4 p. p. m. of nitric nitrogen and 120 p. p. m. of chlorin.

Sample No. 94.—A short distance from the orchard described as No. 93 is a large apple orchard where in 1910 six acres of bearing trees were killed and in 1911 ten acres more were so nearly exterminated that the owner grubbed out all of the sixteen acres. Peaches were set out where the apples were removed and when I saw them in September of 1912 they seemed to be thriving. At this time the soil was moist from a recent irrigation and it was brown everywhere, particularly on the sides and crests of the irrigating furrows. Although this condition prevailed in the remainder of the apple orchard, I was able to find only one tree that was burning. In all probability the nitrates either had not become sufficiently concentrated as yet or they had not been washed down to the zone of the feeding roots. My sample was taken from the ridges of three irrigating furrows and included the top four inches. It contained 34 p. p. m. of nitric nitrogen and 420 p. p. m. of chlorin.

Sample No. 95.—This sample consisted of the brown surface half-inch from the irrigating furrows described in the preceding sample. The color varied from the brown of iron rust to dark chocolate. The soil was quite moist and there was no mealy condition beneath the surface crust. It contained 600 p. p. m. of nitric nitrogen and 150 p. p. m. of chlorin. My purpose in taking this sample was to compare the nitrifying efficiency of the surface crust where the nitrates had become concentrated with that of soil from the same source taken to greater depths.

Sample No. 96.—The next sample came from a peach orchard where the owner complained of the peach leaves turning yellow and the immature fruit dropping. We have never had an opportunity to observe the behavior of bearing peach trees when grown where nitrates are excessive and consequently we did not know just what to expect. So far as our records go, they show that both pears and peaches withstand niter very well. This soil was a nice sandy loam; it exhibited no brown color, and, as a subsequent chemical determination showed, the nitrates were not excessive, although somewhat higher than other soils in that vicinity. We have no reason for thinking that the peach trouble mentioned above was caused by the small amount of nitrates present. The sample was collected near one of the affected trees and contained 20 p. p. m. of nitric nitrogen and 170 p. p. m. of chlorin.

Sample No. 97.—The next sample came from an apple orchard in

a section of the country where no trouble was ever experienced from high nitrates until 1911. Almost every other part had had its trials but this region appeared to have been favored. As I was driving past this orchard one afternoon in October of 1911, I noticed some thirty trees in one corner that were dying unquestionably from too much niter. These were the only trees affected in this way that could be found any place in the neighborhood. The soil is a clay loam and it was rather moist from a recent shower, so it was difficult to determine the presence of the brown color. I visited the place again in July, 1912, and found that the burning had spread over the entire district embracing, I should judge, eight to ten square miles. Practically everything in the way of apple trees was suffering. The owner of the place told me that the burning had all developed since the last irrigation which was begun on Sunday, June 30th, and was continued until the following Wednesday. About three days after he took the water off, he had observed the dark brown stain on the irrigating furrows and in about one week the trees began to burn. That is to say, one week was required for the nitrates, which were carried from the surface to the feeding zone of the apple roots, to produce the physiological effect on the foliage which we have designated as burning.

The soil was hard and dry at the time the sample was taken; there was no brown color visible and judging from the physical condition of the soil, it had received little cultivation and irrigation during the summer and fall of 1912. Nitric nitrogen amounting to 20 p. p. m. and chlorin to 65 p. p. m. were found.

FOREIGN SOILS

Sample No. 52.—Ohio. Received March, 1913; clay loam; moist; corn field; nitric nitrogen 7 p. p. m.; chlorin 8 p. p. m.

Sample No. 53.—Georgia. Received March 10, 1913; red gravelly loam; oak and pine forest; wet when sent; nitric nitrogen 5 p. p. m.; chlorin 78 p. p. m.

Sample No. 54.—Washington. Received March 9, 1913; dark loam; upland soil from an orchard; typical of the river bench soil common to that locality; moisture good; nitric nitrogen 5 p. p. m.; chlorin 40 p. p. m.

Sample No. 55.—Virginia. Received March 10, 1913 heavy clay from an orchard; sample wet due to heavy rains at the time soil was collected; nitric nitrogen 9 p. p. m.; chlorin 74 p. p. m.

Sample No. 56.—Oklahoma. Received March 17, 1913; sandy loam; cotton in 1912, dry; nitric nitrogen 11 p. p. m.; chlorin 8 p. p. m.

Sample No. 57.—North Carolina. Received March 19, 1913; light sandy loam; sharp sand with little organic matter; water melons 1912; moisture good; nitric nitrogen 7 p. p. m.; chlorin 10 p. p. m.

Sample No. 58.—Texas. Received March 18, 1913; dark sandy

loam; never received commercial fertilizer or stable manure; cotton 1912; corn 1911; nitric nitrogen 7 p. p. m.; chlorin 98 p. p. m.

Sample No. 59.—Georgia. Received March 21, 1913; red clay loam; cotton for last three years with average yield of four hundred pounds per acre; nitric nitrogen 14 p. p. m.; chlorin 106 p. p. m.

Sample No. 60.—North Carolina. Received March 22, 1913; light colored clay; planted to corn for several years, gave an average yield of forty to fifty bushels per acre; no manure or fertilizer applied; said to be very good corn land when properly cared for; sample was very dry when received; nitric nitrogen 6 p. p. m.; chlorin 8 p. p. m.

Sample No. 61.—North Carolina. Received March 19, 1913; white sandy loam containing a great deal of sharp white sand; very little organic matter; tobacco in 1912; wheat in 1913; wet; nitric nitrogen 3 p. p. m.; chlorin 6 p. p. m.

Sample No. 62.—California. Received March 8, 1913; clay loam with some adobe; no white alkali on surface; moist; grapes for past few years; nitric nitrogen 4 p. p. m.; chlorin 26 p. p. m.

Sample No. 63.—California. Received March 8, 1913; sandy loam; moist, alfalfa field, no white alkali on surface and no brown color; nitric nitrogen 4 p. p. m.; chlorin 8 p. p. m.

Sample No. 64.—California. Received March 8, 1913; sandy loam; moist; in small grain 1912. No white alkali; nitric nitrogen 6 p. p. m.; chlorin 8 p. p. m.

Sample No. 65.—Arkansas. Received March 28, 1913; red sandy loam from Arkansas bottom; cotton in 1912, soil very wet when collected, so it was dried before sent; nitric nitrogen 4 p. p. m.; chlorin 62 p. p. m.

Sample No. 66.—Oklahoma. Received March 25, 1913; clay loam; sample taken to depth of six inches; alfalfa in 1911, cane in 1912. The person who sent the sample states that it has always been difficult to get trees to grow on this land; nitric nitrogen 40 p. p. m.; chlorin 6 p. p. m.

Sample No. 67.—California. Received March 25, 1913; heavy silt loam; white alkali abundant as an incrustation where the sample was taken; no brown color; moist; no irrigation; young mixed orchard; nitric nitrogen 120 p. p. m.; chlorin 700 p. p. m.

Sample No. 68.—California. Received March 25th, 1913; light sandy loam, the prevailing soil in this region; no white alkali; sugar-beets in 1911, barley in 1912, and set to plums in 1913; nitric nitrogen 7.4 p. p. m.; chlorin 8 p. p. m.

Sample No. 69.—California. Received March 28, 1913; virgin soil from foothills; surface removed to avoid contamination due to wash;

clay; moist; no white alkali; general mountainous vegetation; nitric nitrogen 2 p. p. m.; chlorin 8 p. p. m.

Sample No. 70.—California. Received April 2, 1913; "black adobe"; no white alkali; moist; small grain in 1912, nitric nitrogen 5 p. p. m.; chlorin 10 p. p. m.

Sample No. 71.—California. Received March 7, 1913; sand and gravel from raw cactus land; moist; no white alkali and no brown color; nitric nitrogen, none; chlorin 6 p. p. m.

Sample No. 73.—California. Received April 2, 1913; sandy loam; no white alkali; no brown color; moist; formerly a vineyard; small grain in 1912; nitric nitrogen 7.4 p. p. m.; chlorin 8 p. p. m.

Sample No. 74.—Garden City, Kansas. Received April 17, 1913; sandy loam; sugar-beets for past two years, 1911 and 1912; nitric nitrogen 26 p. p. m.; chlorin 2,000 p. p. m.

DISCUSSION OF RESULTS.

Colorado Soils.

SERIES I. AMMONIUM SULPHATE

In Table No. 1, will be found the results of our nitrification experiments with Colorado soils and ammonium sulphate. Of the twenty-three soils under study, 17 showed a very marked net gain in nitric nitrogen over the checks; one gave the same increase as the corresponding soil which received no ammonium sulphate and therefore is considered as having given no net gain (1); five contained less than in the beginning. The point of greatest interest in this series is the uniformly large gains which all of the positive soils made. There are no small increases of 10, 20, or 30 parts per million as might be expected, the smallest being 320. Furthermore, there is some justification for setting aside this figure as the lowest since the sample consisted of the surface crust from a brown area and does not represent a four to six inch section of soil as the others do. The nitric nitrogen was high to begin with, 600 p. p. m., and that may have had some retarding influence on the rate of nitrification. If No. 95 is eliminated, then we have the lowest net gain produced by any positive soil as 408 p. p. m. by No. 94, while the average is 672 and the maximum 972.

As a matter of interest in connection with Nos. 94 and 95, the reader's attention is called to the initial nitrate nitrogen in the two samples. It will be remembered that both were taken side by side, but that the former represents a soil-section a short distance from any brown material while the latter is just the brown surface; the first carried 34 p. p. m. and the second 600. The chlorin is considerably lower in the crust than in the section so it is probably the high nitrates that are responsible for the relatively small increase in nitric nitrogen.

(1) By the expression "total gain" is meant the increase in nitric nitrogen over the check at the beginning; by "net gain" is meant the increase over the check at the end.

Considering only those soils which gave positive results, there seems to be practically no difference in the activity of our normal and incipient niter soils as measured by their ability to nitrify ammonium sulphate

Looking next to the samples that show either no gain or a loss of nitric nitrogen, we find that with the exception of No. 78, the raw

TABLE NO. 1.—Nitrification of Ammonium Sulphate by Colorado Soils. Nitrogen in Parts Per Million of Air-dried Soil as Nitrites and Nitrates from 100 m.g. Nitrogen as $(\text{NH}_4)_2\text{SO}_4$. Duration of Experiment: 6 Weeks at 28 Degrees C.

Number of Sample and Description.	Nitrogen as nitrites in parts per million			Nitrogen as nitrates in parts per million					Chlorin in P. P. M.
	Check at begin.	Check at end	$(\text{NH}_4)_2\text{SO}_4$ added	Check at begin.	Check at end	$(\text{NH}_4)_2\text{SO}_4$ added	Total gain	Net gain	
75 Wheat field, clay, normal	.88	.36	1.60	7.	64.	800.	793.	736.	500.
76 Truck garden, river bottom, loam, normal	1.20	.002	.92	54.	110.	660.	606.	550.	400.
77 Oat field, clay loam, niter	.32	.26	2.00	26.	76.	800.	774.	724.	4,500.
78 Raw land, adobe hill	.36	.60	25.00	140.	140.	108.	—32.	—32.	700.
79 Apple orchard, red clay loam, niter	.26	.60	9.60	5.	66.	600.	595.	534.	1,600.
80 Apple orchard, clay loam, niter	.28	.90	68.00	13.	50.	36.	23.	—14.	10,000.
81 Barren field, brown crust, niter	.32	.22	1.60	1360.	1360.	1240.	—120.	—120.	32,400.
82 Barren field, clay, niter	1.60	.80	1.60	46.	120.	660.	614.	540.	9,800.
83 Apple orchard, heavy clay, niter	.02	.36	2.40	6.	100.	1040.	1034.	940.	38.
84 Apple orchard, heavy clay, niter	.14	.52	1.00	Trace	68.	1040.	1040.	972.	138.
85 Apple orchard, clay loam, niter	.012	.10	2.00	6.	60.	600.	594.	540.	1,000.
86 Oat field, sandy loam, niter	.0	.14	.10	130.	240.	200.	70.	—40.	9,400.
87 Apple orchard, clay loam, niter	.10	.12	.60	6.	40.	840.	834.	800.	200.
88 Apple orchard, clay loam, niter	.40	.14	.80	70.	100.	100.	30.	0.	8,300.
89 Apple orchard, clay loam, niter	.40	.60	.14	180.	260.	800.	620.	540.	1,600.
90 Apple orchard, sandy loam, niter	.32	.40	.36	13.	60.	800.	867.	820.	140.
91 Apple orchard, heavy clay, normal	.32	.14	.10	Trace	40.	720.	720.	680.	80.
92 Oat field, sandy loam, niter	1.40	.20	.36	320.	320.	240.	—80.	—80.	7,800.
93 Peach orchard, heavy clay, normal	.60	.60	2.40	4.	48.	880.	876.	832.	120.
94 Apple orchard, clay niter	.14	1.20	.10	34.	92.	500.	466.	408.	420.
95 Apple orchard, brown surface	.26	.14	.60	600.	640.	960.	360.	320.	150.
96 Peach orchard, sandy loam, normal	.24	1.00	2.40	20.	60.	720.	700.	660.	170.
97 Apple orchard, clay niter	.36	1.40	.24	20.	132.	960.	940.	828.	65.

adobe hill, all contain very high chlorin; in fact with the exception of No. 82, which gave positive results, the five soils with the highest chlorin are the negative soils. Lipman (1) has shown experimentally that when sodium chlorid is present in soil in amounts equivalent

(1) Lipman, Chas. B., Toxic Effects of "Alkali Salts" in Soils on Soil Bacteria. II. Nitrification. Cent. f. Bakt., II. Abt., Bd. 33, p. 305, 1912.

to .2 per cent. of the dry soil, that a very marked toxic effect is produced upon the nitrifying organisms; and that when a concentration of .4 per cent. is reached, no nitrification takes place. This seems to be the most plausible explanation of the failure of our high chlorin soils to produce nitrate. The chlorin in these, when computed as NaCl, gives the following concentrations which fall easily beyond the limits of tolerance as established by Lipman; No. 80, 1.65 per cent.; No. 81, 5.34 per cent.; No. 86, 1.05 per cent.; No. 88, 1.36 per cent.; No. 92, 1.30 per cent. While this accounts vary nicely for the lack of nitrifying efficiency on the part of five samples, it makes it very difficult to explain why No. 82 with 9,800 p. p. m. of chlorin or 1.61 per cent. NaCl should respond positively with a net gain of 540 p. p. m. of nitric nitrogen.

On the other hand, Professor Lipman has shown that the presence of .05 per cent and even .1 per cent NaCl has a stimulating effect upon nitrification. Almost all of our positively reacting soils contain moderate amounts of sodium chlorid, varying from .009 per cent. to .165 per cent. and it is not at all improbable that the chlorin acts as an intensifying agent to nitrification.

The lack of nitrifying efficiency shown by No. 78 can hardly be accounted for by the presence of high chlorids, since it carries considerably less than several of the more efficient samples. The high nitrites which are present, 25 p. p. m., seem to indicate a vigorous denitrifying flora stimulated by the sulphate, rather than retarded nitrification. This follows from the fact that the control increased but very little, .24 p. p. m., in nitrous nitrogen and none at all in nitric nitrogen, while with ammonium sulphate added, there was a loss of nitrates and an appreciable increase of nitrites. Are we to infer from this that the $(\text{NH}_4)_2\text{SO}_4$ has favored the reducing agents? The failure of the control to increase in nitrogen would seem to support the view that the nitrifying organisms were either absent or the conditions were very unfavorable for their development. What has been said here applies equally well to No. 92, except that the high chlorin may account for the failure of the control to increase in nitrates.

The conditions were somewhat different in No. 80. Here we have evidence in the increased nitric nitrogen of the control that the nitrifying organisms were moderately vigorous; however, the addition of the ammonium sulphate has retarded their activity to a very marked degree as indicated by the 68 p. p. m. of nitrite nitrogen and 23 p. p. m. of nitrate nitrogen as compared with .9 p. p. m. and 50 p. p. m. respectively in the control.

Any interpretation that we might give to No. 86 would be little more than mere conjecture, other than to state that something has either interfered with nitrification or that denitrification has been exceedingly rapid in the presence of the ammonium salt. In either case, we should expect to find the nitrates higher than they are. That the nitrifying organisms are present there is no question for the control shows a material gain.

In No. 88, we have nitrification going on in the control but rather feebly. There was no increase in the nitric nitrogen with the additional supply of ammonia nitrogen. Knowing the condition and history of this soil, I am inclined to explain this by a loss of vitality and virulence on the part of the organisms rather than by their inability to attack ammonium sulphate. The water soluble salts in this soil were very high and the nitrifying bacteria were, in all probability, intoxicated, so to speak, by the saline solution.

SERIES II. AMMONIUM CHLORID

The results of the next series are given in Table No. 2. Here the ammonia nitrogen was furnished in the form of ammonium chlorid. A

TABLE NO. 2.—Nitrification of Ammonium Chlorid by Colorado Soils. Nitrogen in Parts Per Million of Air-dried Soil as Nitrites and Nitrates from 100 mg. Nitrogen as NH_4Cl
Duration of Experiment: 6 Weeks at 28 Degrees C.

Number of Sample and Description	Nitrogen as nitrites in parts per million			Nitrogen as nitrates in parts per million				Total gain Net gain		Chlorin in p. p. m.
	Check at begin.	Check at end	NH_4Cl added	Check at begin.	Check at end	NH_4Cl added				
83 Apple orchard, heavy clay, niter02	.36	.06	6.	100.	120.	114.	20.	58.	
84 Apple orchard, heavy clay, niter14	.52	.10	Trace	68.	320.	320.	252.	138.	
85 Apple orchard, clay loam, niter012	.10	.20	6.	60.	12.	6.	—48.	1,000.	
86 Oat field, sandy loam, niter00	.14	.04	130.	240.	160.	30.	—80.	6,400.	
87 Apple orchard, clay loam, niter10	.12	.072	6.	40.	32.	26.	—8.	202.	
88 Apple orchard, clay loam, niter40	.14	.02	70.	100.	100.	30.	0.	8,300.	
89 Apple orchard, sandy loam, niter40	.60	.08	180.	260.	200.	20.	—60.	1,600.	
90 Apple orchard, sandy loam, niter32	.40	.10	13.	60.	120.	107.	60.	140.	
91 Apple orchard, heavy clay, normal32	.14	.112	Trace	40.	72.	72.	32.	80.	
92 Oat field, sandy loam niter	1.40	.20	.18	320.	320.	280.	—40.	—40.	7,500.	
93 Peach orchard, heavy clay, normal60	.60	.112	4.	48.	120.	116.	72.	120.	
94 Apple orchard, clay niter14	1.20	.10	34.	92.	240.	206.	148.	420.	
95 Apple orchard, brown surface26	.14	.06	600.	640.	680.	80.	40.	150.	
96 Peach orchard, sandy loam, normal24	1.00	.12	20.	60.	96.	76.	36.	170.	
97 Apple orchard, clay, niter36	1.40	.26	20.	132.	560.	540.	428.	65.	

glance at the net gain column of this table brings out the striking fact that ammonium chlorid does not begin to be as fertile a source of nitric nitrogen as the sulphate. Whether the additional chlorid has exercised an inhabiting action or whether this form of the salt is more resistant to the attacks of the nitrifying organisms is difficult to say. Nine of the fifteen samples gave a positive gain in nitric nitrogen with the chlorid; one showed no increase and five failed to produce as much as the controls. None yielded as much as the respective soils in the sulphate series, and all that fell behind here were also below in the preceding set. All of the negative results point strongly to the inhibi-

tion of the nitrifying organisms rather than to denitrification. The net gains in nitric nitrogen varied from 2.13 per cent. to 51.69 per cent. of those secured from ammonium sulphate with the corresponding soils; compared with the returns from ammonium carbonate, the third series, the gains varied from 3.49 per cent. to 37.28 per cent.; with dried blood, the fourth set, they varied from 4.00 per cent. to 57.22 per cent. The average net gain in nitrate nitrogen for the positive soils was 120.88 p. p. m., with the maximum of 428.0 p. p. m. and a minimum of 20 p. p. m.

SERIES III.. AMMONIUM CARBONATE

Ammonia nitrogen was supplied in the form of ammonium carbonate to the third set of samples. The results of the study appear in

TABLE NO. 3.—Nitrification of Ammonium Carbonate by Colorado Soils. Nitrogen in Parts Per Million of Air-dried Soil as Nitrites and Nitrates from 100 m.g. Nitrogen as $(\text{NH}_4)_2\text{CO}_3$, Duration of Experiment: 6 Weeks at 28 Degrees C.

Number of Sample and Description	Nitrogen as nitrites in parts per million			Nitrogen as nitrates in parts per million			Total gain	Net gain	Chlorin in p. p. m.
	Check at begin.	Check at end	$(\text{NH}_4)_2\text{CO}_3$ added	Check at begin.	Check at end	$(\text{NH}_4)_2\text{CO}_3$ added			
75 Wheat field, clay, normal	.88	.36	.152	7.	64.	720.	713.	656.	500.
76 Truck garden, river bottom loam, normal	1.20	.002	.08	54.	110.	680.	626.	570.	400.
77 Oat field, clay loam, niter	.32	.26	.08	26.	76.	600.	574.	524.	4,500.
78 Raw land, adobe hill	.36	.60	.04	140.	140.	100.	—40.	—40.	700.
79 Apple orchard, red clay loam, niter	.26	.60	.32	5.	66.	560.	555.	494.	1,000.
80 Apple orchard, clay loam, niter	.28	.90	48.00	13.	50.	36.	23.	—14.	10,000.
81 Barren field, brown crust, niter	.32	.22	.60	1360.	1360.	1240.	—120.	—120.	32,400.
82 Barren field, clay niter	1.60	.80	.20	46.	120.	700.	654.	580.	9,800.
83 Apple orchard, heavy clay, niter	.02	.36	.20	6.	100.	672.	666.	572.	58.
84 Apple orchard, heavy clay, niter	.14	.52	.02	Trace	68.	700.	700.	632.	138.
85 Apple orchard, clay loam, niter	.012	.10	3.60	6.	60.	400.	394.	340.	1,000.
86 Oat field, sandy loam, niter	.0	.14	.06	130.	240.	200.	70.	—40.	6,400.
87 Apple orchard, clay loam, niter	.1	.12	5.60	6.	40.	340.	334.	300.	202.
88 Apple orchard, clay loam, niter	.40	.14	.80	70.	100.	100.	30.	0.	8,300.
89 Apple orchard, sandy loam, niter	.40	.60	.12	180.	260.	600.	420.	340.	1,600.
90 Apple orchard, sandy loam, niter	.32	.40	.40	13.	60.	660.	647.	600.	140.
91 Apple orchard, heavy clay, normal	.32	.14	.32	Trace	40.	700.	700.	660.	80.
92 Oat field, sandy loam niter	1.40	.20	.10	320.	320.	340.	20.	20.	7,800.
93 Peach orchard, heavy clay, normal	.60	.60	1.40	4.	48.	640.	634.	592.	120.
94 Apple orchard, clay niter	.14	1.20	9.60	34.	92.	700.	666.	608.	420.
95 Apple orchard, brown surface	.26	.14	.12	600.	640.	1320.	720.	680.	150.
96 Peach orchard, sandy loam, normal	.24	1.00	8.00	20.	60.	120.	100.	60.	170.
97 Apple orchard, clay, niter	.36	1.40	1.00	20.	132.	1280.	1260.	1148.	63

Table No. 3. They indicate that this compound of ammonium is broken down with ease compared with the ammonium chlorid, but not quite as readily as the sulphate. Eighteen of our soils gave positive gains in nitric nitrogen from the carbonate; one showed no increase and four produced less than the controls. We find the samples which failed to nitrify the carbonate the same four that fell below with the sulphate, and what has been said by way of an attempted explanation of this under the sulphate series should hold equally well here. With the exception of No. 78, all are high in chlorin. A few points of interest in connecting with these negatively reacting soils may be mentioned here: No. 78, the raw adobe hill, failed by 8 p. p. m. to produce as much nitric nitrogen in the presence of the carbonate as of the sulphate; the vigorous denitrification appears to have carried the nitrates beyond the nitrite stage since the nitrous nitrogen is less than in the beginning. We have a rather striking coincidence in the exact agreement of soils 80, 81 and 86 with respect to the nitric nitrogen that they produced in the presence of both ammonium carbonate and ammonium sulphate. No. 92, which failed to give a net gain in nitric nitrogen with $(\text{NH}_4)_2\text{SO}_4$ yielded an increase of 20 p. p. m. over the control with $(\text{NH}_4)_2\text{CO}_3$, and this with 7,800 p. p. m. of chlorin present.

The average net gain in nitric nitrogen made by the positively reacting soils was 520.88 p. p. m., the maximum was 1148.0 p. p. m. by soil No. 97, and the minimum 20 p. p. m. by No. 92.

The results of this study indicate that both our normal and incipient niter soils are equally efficient in nitrifying ammonium carbonate.

The presence of large quantities of initial nitrate in the soil without high chlorin such as we find in No. 95 with 600 p. p. m. of nitric nitrogen did not interfere seriously with the nitrification process. In this particular instance, a net gain of 680 p. p. m. of nitric nitrogen was secured, although the sample to begin with contained a large excess, it being the brown crust from a niter spot.

SERIES IV. DRIED BLOOD

As stated elsewhere, we were curious to learn whether the ammonium compounds that are formed by the ammonification of proteid nitrogen, through the agency of ammonifying bacteria, would respond more or less readily to the nitrifying organisms than would the chemically pure salts. To this end, a fourth series of soils was prepared, to which dried blood was added to furnish the necessary organic nitrogen.

The results of the experiment appear in Table No. 4. In the previous investigation, we determined the ammonifying efficiency of soils from all of these localities except three and found them to be abundantly stocked with ammonifying organisms and capable of converting the nitrogen of the blood into ammonia nitrogen. Having ascertained this fact, we have proceeded on the assumption, in the present work, that ammonium compounds would be available for the nitrifying organisms, and that if there was no increase in nitrate at the end of the experimental period, it was chargeable to the nitrifying flora and not to the ammonifiers.

The addition of the dried blood to the soil appears to have improved the condition for nitrification in several of the samples for twenty of them gave gains in nitric nitrogen above the control; one produced the same and two less. It would be interesting to know whether the increased nitrifying efficiency in these cases has resulted from the additional food material or whether the deleterious action of the high chlorids has been lessened by some combination with the blood.

TABLE NO. 4.—Nitrification of Dried Blood by Colorado Soils. Nitrogen in Parts Per Million of
of Air-dried Soil as Nitrites and Nitrates from 100 m.g. Nitrogen as Dried Blood
Duration of Experiment: 6 Weeks at 28 Degrees C.

Number of Sample and Description	Nitrogen as nitrites in parts per million			Nitrogen as nitrates in parts per million					Chlorin in p. p. m.
	Check at begin.	Check at end	Dried Blood added	Check at begin.	Check at end	Dried Blood added	Total gain	Net gain	
75 Wheat field, clay, nor- mal88	.36	.30	7.	64.	680.	673.	616.	500.
76 Truck garden, river bottom loam, normal	1.20	.002	.26	54.	110.	680.	626.	570.	400.
77 Oat field, clay loam, niter32	.26	.88	26.	76.	600.	574.	524.	4,500.
78 Raw land, adobe hill36	.60	.64	140.	140.	140.	0.	0.	700.
79 Apple orchard, red clay loam, niter26	.60	1.00	5.	66.	640.	635.	590.	1,000.
80 Apple orchard, clay loam, niter28	.90	88.00	13.	50.	100.	87.	50.	10,000.
81 Barren field, brown crust, niter32	.22	.84	1360.	1360.	740.	—620.	—620.	32,400.
82 Barren field, clay niter	1.60	.80	1.70	46.	120.	500.	454	380.	9,800.
83 Apple orchard, heavy clay, niter02	.36	.08	6.	100.	600.	594.	500.	58.
84 Apple orchard, heavy clay, niter14	.52	.18	Trace	68.	880.	880.	812.	138.
85 Apple orchard, clay loam, niter012	.10	.26	6.	60.	620.	614.	560.	1,000.
86 Oat field, sandy loam, niter00	.14	.02	130.	240.	80.	—50.	—160.	6,400.
87 Apple orchard, clay loam, niter10	.12	35.00	6.	40.	320.	314.	280.	202.
88 Apple orchard, clay loam, niter40	.14	.60	70.	100.	240.	170.	140.	8,300.
89 Apple orchard, sandy loam, niter40	.60	5.20	180.	260.	480.	300.	220.	1,600.
90 Apple orchard, sandy loam, niter32	.40	.60	13.	60.	600.	587.	540.	140.
91 Apple orchard, heavy clay, normal32	.14	8.80	Trace	40.	440.	440.	400.	80.
92 Oat field, sandy loam niter	1.40	.20	.192	320.	320.	340.	20.	20.	7,800.
93 Peach orchard, heavy clay, normal60	.60	40.00	4.	48.	240.	236.	192.	120.
94 Apple orchard, clay niter14	1.20	4.80	34.	92.	400.	366.	308.	420.
95 Apple orchard, brown surface26	.14	.08	600.	640.	1000.	400.	360.	150.
96 Peach orchard, sandy loam, normal24	1.00	.32	20.	60.	880.	860.	820.	170.
97 Apple orchard, clay, niter36	1.40	1.20	20.	132.	880.	860.	748.	65.

No. 78, from the raw adobe hill, presents an interesting case again in this series. It will be remembered that in two preceding studies, Series I and III, this soil showed active denitrification in the presence of the different nitrifiable substances, and that the controls contained the same amount of nitrate at the end of the experimental period as in the beginning. Here, in place of the reduction of its initial nitrates, it has

maintained them constant to the end, and while it can show no net gain in nitric nitrogen, it has not lost what it already had, as before.

In soil No. 80, Series I and III, nitrification seems to have been retarded by the presence of the ammonium salts, due probably to combinations of chlorids with the sulphates or carbonates or perhaps to mere concentration of the saline solution. The dried blood in the present series has evidently changed the soil conditions quite materially, for here No. 80 gave a net gain of 50 p. p. m. nitric nitrogen and 88. p. p. m. of nitrous nitrogen over the control, whereas in both preceding studies it had shown a loss in the former but a gain in the latter due presumably to denitrification.

Soil No. 88, which gave no gains in any of the preceding experiments, responded to the dried blood with a net increase of 140 p. p. m. of nitric nitrogen.

Samples Nos. 81 and 86 were unable to utilize the dried blood in the manufacture of nitrates. These same soils, it will be remembered, were deficient in nitrifying efficiency when tested out with the ammonium salts.

No. 81 showed the largest loss in nitric nitrogen, 620 p. p. m., of any soil in any series.

No. 92, which gave no increase with the sulphate and chlorid, showed a gain of 20 p. p. m. here as with the carbonate.

The maximum net gain in nitrogen was made by No. 96 with 820 p. p. m. This figure is rather interesting in view of the fact that the yields of this soil from both ammonium carbonate and ammonium chlorid were among the lowest. The average gain for the positively reacting soils of this series was 431.50 p. p. m., and the minimum 20 p. p. m. by No. 92.

The presence of large quantities of nitrate in the soil to begin with does not appear to interfere seriously with the nitrification of dried blood provided the chlorin is not also high; this is apparent from the results of samples Nos. 81 and 95. Comparing the nitrifying efficiency of our positively reacting normal soils with that of our incipient niter soils, we find that in this series the normal samples have considerably the advantage; the normal gave an average gain of 519.60 p. p. m. of nitric nitrogen against 405.14 p. p. m. for the niter.

SUMMARY OF COLORADO SOILS

Seventeen or 73.91 per cent. of the soils under examination were able to convert the nitrogen of ammonium sulphates into nitrate nitrogen; eighteen or 78.26 per cent. accomplished the same with ammonium carbonate and twenty or 86.95 per cent. with dried blood. Of the fifteen that received ammonium chlorid, nine or 60 per cent. gave positive results.

The largest total amount of nitric nitrogen was produced from ammonium sulphate; the next largest from the ammonium carbonate, then the dried blood and least from the ammonium chlorid.

The largest amount of nitric nitrogen produced by any one soil

from ammonium sulphate was 972 p. p. m. by No. 84., a heavy clay niter soil; from ammonium chlorid, it was 428 p. p. m. by No. 97, a clay niter soil; from ammonium carbonate, it was 1148 p. p. m. by No. 97; from dried blood, it was 820 p. p. m. by No. 96, a sandy loam, normal soil.

The average amount of nitric nitrogen produced by a positively reacting soil in each series was as follows: Ammonium sulphate, 672 p. p. m.; ammonium carbonate, 520.88 p. p. m.; dried blood, 431.50 p. p. m.; ammonium chlorid, 120.88 p. p. m.; check 59.30 p. p. m.

The average amount of nitric nitrogen produced by a Colorado soil in the different series, all samples taken into consideration, positive and negative, was as follows: Ammonium sulphate, 484.26 p. p. m.; ammonium carbonate, 398.34 p. p. m.; dried blood, 341.30 p. p. m.; ammonium chlorid, 56.80 p. p. m.; check, 51.30 p. p. m.

The average amount of nitric nitrogen produced by a niter soil in the incipient stage, and a normal soil in each series was as follows: Ammonium sulphate, 710.6 p. p. m. for the nitre, 691.6 p. p. m. for the normal; ammonium chlorid 181.6 p. p. m. for the niter, 46.66 p. p. m. for the normal; ammonium carbonate, 507.6 p. p. m. for the niter and 505.6 p. p. m. for the normal; dried blood, 405.14 for the niter, 519.6 for the normal. The differences in the average gain from the sulphate and the carbonate are so small, 19 p. p. m. for the former and 2 p. p. m. for the latter, that we are reasonably safe in saying that our incipient niter soils and normal soils are equally efficient in nitrifying these substances. In the case of the dried blood, the normal soils have out-yielded the others by 114.46 p. p. m. per sample. In the ammonium chlorid series, the number of samples under consideration is too small for the results to carry much weight; however, our figures taken for what they may be worth, indicate that the niter soils are superior to the normal, the former yielding an average of 34.94 p. p. m. of nitrate nitrogen more than the latter.

Considering the positively reacting soils, or those which showed an increase in nitric nitrogen above the controls, we find that 52.94 per cent. of them made their largest gains from the ammonium sulphate; 33.33 per cent. from the ammonium carbonate, 30 per cent from the dried blood and none from the ammonium chlorid; 41.17 per cent. made their second highest gains from ammonium sulphate; 38.88 per cent. from the carbonate; 15 per cent. from the dried blood and none from the ammonium chlorid 11.76 per cent. gave the third largest yields from the ammonium sulphate; 27.77 per cent. from the carbonate; 55 per cent. from the dried blood and 0. per cent. from the chlorid of ammonium.

21.74 per cent. of the soils produced less nitric nitrogen in the presence of ammonium sulphate than the controls, while 4.35 per cent. gave the same; 33.33 per cent. gave less with the ammonium chlorid than the checks, and 6.67 per cent. the same; 17.39 per cent. yielded less from ammonium carbonate than the controls and 4.35 per cent. the same; 8.7 per cent. produced less from dried blood than the controls, and 4.35 per cent. the same.

Foreign Soils.

In order that we might have some first-hand data with which to compare the nitrifying efficiency of our niter soils, we have carried side by side with these, twenty-two soils from localities outside of Colorado. An effort has been made throughout the investigation to eliminate all factors that would tend to lessen the value of the comparative study. With the exception of the collection and shipment of the samples, all have been handled by the same workers so that so far as the personal equation and methods of technique are concerned, the results of both series should be fair to each other and comparable.

Ammonium chlorid has been omitted from the list of nitrifiable substances employed in connection with these soils since our experience with the salt and the Colorado samples led us to believe that it possessed little value as a source of nitrogen for measuring nitrifying efficiency.

SERIES V. AMMONIUM SULPHATE.

In table No. 5 will be found the results of the first set of nitrification experiments with foreign soils in which ammonium sulphate was employed as the nitrifiable substance. The relatively low chlorids in these soils stand out in striking contrast to those with which we have just been dealing, and with the exception of Nos. 67 and 74, the chlorin is so low that it can be considered as a negligible quantity.

Seventeen or 77.27 per cent. of the soils gave positive results with the sulphate and five or 22.73 per cent. negative.

Among the samples which failed to produce as much nitric nitrogen as the controls are two, Nos. 57 and 61, which are of particular interest because of their peculiar physical character; both, it will be remembered, are sandy loams, for the most part clean, sharp, white sand, the latter considerably coarser than the former, and very deficient in organic matter. The writer is well acquainted with both soils and can state that the samples are typical of the regions from which they come. It may be of some interest to note in passing that the Fusarium wilt of water melons has been so serious on the first of these that melon growing has been abandoned almost entirely in recent years; the first observations on the Granville tobacco wilt, caused by *B. solanacearum* and described by Stevens(1) and the writer in 1903, were made on the tract from which No. 61 came. The nitrifying organisms seemed to be present in these samples as shown by the gains in the controls, but the ammonium sulphate inhibited their action almost completely; in fact, in No. 57, it checked it altogether, and a little reduction took place, while in the other sample, there was a gain of 1 p. p. m. nitric nitrogen over the initial content, but still less than the control.

Nos. 58, 59, and 60 all showed the retarding effect of the sulphate on nitrification. That the necessary organisms were present to transform the ammonium nitrogen into nitrate nitrogen is clearly evident for all of the controls gave very appreciable gains.

(1) Stevens, F. L., and Sackett, W. G.; Granville Tobacco Wilt, Bul. 188, N. Carolina Exp. Sta., 1903.

A peculiar condition existed in No. 53 throughout the investigation; the check lost practically all of its nitric nitrogen, but the addition of the ammonium sulphate appeared to have retarded denitrification so that the soil to which this salt was added showed an excess of 3 p. p. m. nitric nitrogen over the control, but at the same time a loss of 2 p. p. m. of its initial nitrate nitrogen. Although it contained more nitric nitrogen than the check at the end of the experiment, it might be grouped more properly with the negatively reacting soils, since the nitric nitrogen which was found was in all probability, a part of the initial content which had not yet been reduced.

TABLE NO. 5.—Nitrification of Ammonium Sulphate by Foreign Soils. Nitrogen in Parts Per Million of Air-dried Soil as Nitrites and Nitrates from 100 m.g. Nitrogen as $(\text{NH}_4)_2\text{SO}_4$, Duration of Experiment: 6 Weeks at 28 Degrees C.

Number of Sample and Description	Nitrogen as nitrites in parts per million			Nitrogen as nitrates in parts per million					Chlorin in p. p. m.
	Check at begin.	Check at end	$(\text{NH}_4)_2\text{SO}_4$ added	Check at begin.	Check at end	$(\text{NH}_4)_2\text{SO}_4$ added	Total gain	Net gain	
52 Corn field, clay loam, Ohio002	.176	1.20	7.	60.	120.	113.	60.	8.
53 Pine forest, gravelly loam, Georgia	Trace	.220	.44	5.	Trace	3.	—2.	3.	78.
54 Apple orchard, loam, Washington04	.340	.68	5.	90.	120.	115.	30.	40.
55 Apple orchard, heavy clay, Virginia	Trace	.160	.80	9.	70.	240.	231.	140.	74.
56 Cotton field, sandy loam, Oklahoma ..	.18	.168	.80	11.	50.	70.	59.	20.	8.
57 Water melons, sandy loam, North Carolina ..	Trace	.168	.80	7.	46.	6.	—1.	—40.	10.
58 Cotton field, sandy loam, Texas12	.160	.52	7.	50.	30.	23.	—20.	98.
59 Cotton field, clay loam, Georgia	Trace	.152	.80	14.	60.	30.	16.	—30.	106.
60 Corn field, clay, North Carolina16	.112	1.00	6.	70.	40.	34.	—30.	8.
61 Tobacco field, sandy loam, N. Carolina ..	Trace	.100	.52	3.	20.	4.	1.	—16.	6.
62 Vineyard, clay loam, California	Trace	Trace	.48	4.	24.	140.	136.	116.	26.
63 Alfalfa field, sandy loam, California ..	.04	.100	1.20	4.	46.	140.	136.	94.	8.
64 Small grain, sandy loam, California ..	.88	.320	1.20	6.	70.	200.	194.	130.	8.
65 Cotton field, sandy loam, Arkansas60	.132	.60	4.	24.	480.	476.	450.	62.
66 Cane field, clay loam, Oklahoma18	.136	.80	40.	126.	180.	140.	54.	6.
67 Mixed orchard, silt loam, California	1.80	1.80	24.00	120.	140.	280.	160.	140.	700.
68 Plum orchard, sandy loam, California26	.140	1.40	7.4	40.	100.	92.6	60.	8.
69 Virgin soil, clay, California	Trace	.100	1.00	2.	30.	100.	98.	70.	8.
70 Small grain, black adobe, California	1.40	.160	1.40	5.	34.	560.	555.	526.	10.
71 Raw cactus land, sand and gravel, Calif.	Trace	.120	.64	0.	12.	13.	13.	1.	6.
73 Vineyard, sandy loam, California	0.0	.120	.80	7.4	70.	100.	92.6	30.	8.
74 Sugar beets, sandy loam, Kansas	1.2	.260	1.60	26.0	160.	800.	77.4	640.	2,000

The raw cactus land from California, No. 71, was very deficient in nitrifying organisms, while the virgin clay soil, No. 69, gave a net gain of 70 p. p. m. nitric nitrogen.

The largest gains in the series were made by the soils from Ar-

kansas, California(1) and Kansas, Nos. 65, 70 and 74, amounting to 450, 526 and 640 p. p. m. respectively. I was somewhat surprised to find No. 65 so active, but disappointed at not getting larger returns from more of the California samples, since I expected them to behave more like the Colorado soils. The large gain in the Kansas sample fulfilled my expectations since it came from just across the state line and is in all respects a Colorado soil.

The nitrites which appear in connection with No. 67, 24 p. p. m., suggest retarded nitrification rather than denitrification. The 700 p. p. m. of chlorin which the soil contained may have had something to do with this, although No. 74 with 2000 p. p. m. exhibits no such quantity of nitrites.

The maximum net gain in nitric nitrogen from ammonium sulfate was made by No. 74, with 640 p. p. m. As mentioned before, this is essentially a Colorado soil, and it is hardly fair to the other samples to give it first place. The next best increase 520 p. p. m. is shown by No. 70, California black adobe. The average gain for all of the positively reacting soils of this series was 150.82 p. p. m. nitric nitrogen, and the minimum, 1 p. p. m. by No. 71.

SERIES VI. AMMONIUM CARBONATE

Ammonium carbonate was supplied to the soils of this series as the nitrifiable substance. The detailed results appear in Table 6.

Of the twenty-two samples under study, seventeen or 77.27 per cent. made gains in nitric nitrogen from the carbonate of ammonium, while five or 22.73 per cent. failed to do so. Although the number of samples reacting positively and negatively is the same here as in the preceding set, the same soils have behaved differently. Whereas Nos. 58, 59 and 60 were negative with respect to the sulphate, they are positive in the present series. The most striking difference between the results of the two series is in the increased number of soils that gave large net gains; with ammonium carbonate six responded strongly, as compared with three with the ammonium sulphate. Two of these Nos. 70 and 74, are the same as in Series V, but the other four are all different. Three of them are from California, one from Kansas, one from North Carolina and one from Virginia. The natural inference from such an increased yield of nitric nitrogen is that the ammonium carbonate is more easily nitrified than the sulphate, so far as these particular soils are concerned.

Nos. 57, 61, 62, 63, and 71 all produced less nitrate nitrogen than the controls. In No. 57, there was marked inhibition of nitrification in the presence of the carbonate with a very little reduction. Considerable nitrification took place in the check. Nos. 61, 62 and 71 show that the same conditions existed without the denitrification. With No. 63, the nitrifying activities were not retarded to nearly as great a degree, the check producing only 6 p. p. m. more nitric nitrogen than

(1) No. 70 from California compared very favorably in nitrifying efficiency with the Colorado soils in all of the series.

the carbonate sample. All of these negatively reacting soils contained the necessary nitrifying organisms as manifested by the appreciable gains that the checks made. There seems to be little doubt in the case of the five soils just mentioned that the ammonium carbonate has exercised some toxic action, thus preventing the normal development of the nitrifying flora.

The ammonium carbonate appears to have improved conditions for nitrification very much for Sample No. 69, for it gave a net gain of 450 p. p. m. of nitric nitrogen as compared with 70 p. p. m. in the preceding series.

TABLE NO. 6.—Nitrification of Ammonium Carbonate by Foreign Soils. Nitrogen in Parts Per Million of Air-dried Soil as Nitrites and Nitrates from 100 m.g. Nitrogen as $(\text{NH}_4)_2\text{CO}_3$. Duration of Experiment: 6 Weeks at 28 Degrees C.

Number of Sample and Description	Nitrogen as nitrites in parts per million			Nitrogen as nitrates in parts per million					Chlorin in p. p. m.
	Check at begin.	Check at end	$(\text{NH}_4)_2\text{CO}_3$ added	Check at begin.	Check at end	$(\text{NH}_4)_2\text{CO}_3$ added	Total gain	Net gain	
52 Corr. field, clay loam, Ohio002	.176	Trace	7.0	60.	200.	193.	140.	8.
53 Pine forest, gravelly loam, Georgia.....	Trace	.220	.08	5.0	Trace	5.	0.	5.	78.
54 Apple orchard, loam, Washington04	.340	.08	5.0	90.	280.	275.	190.	40.
55 Apple orchard, heavy clay, Virginia	Trace	.160	.02	9.0	70.	320.	311.	250.	74.
56 Cotton field, sandy loam, Oklahoma ..	.18	.168	.04	11.0	50.	240.	229.	190.	8.
57 Water melons, sandy loam, North Carolina	Trace	.168	.14	7.0	46.	6.	—1.	—40.	10.
58 Cotton field, sandy loam, Texas12	.160	Trace	7.0	50.	240.	233.	190.	98.
59 Cotton field, clay loam, Georgia	Trace	.152	Trace	14.0	60.	240.	226.	180.	106.
60 Corn field, clay, North Carolina16	.112	Trace	6.0	70.	360.	354.	290.	8.
61 Tobacco field, sandy loam, N. Carolina...	Trace	.100	.12	3.0	20.	3.	0.	—17.	6.
62 Vineyard, clay loam, California	Trace	Trace	30.00	4.0	24.	4.	0.	—20.	26.
63 Alfalfa field, sandy loam, California ..	.04	.100	26.00	4.0	46.	40.	36.	—6.	8.
64 Small grain, sandy loam, California ..	.88	.320	.32	6.0	70.	160.	154.	90.	8.
65 Cotton field, sandy loam, Arkansas60	.132	30.00	4.0	24.	120.	116.	96.	62.
66 Cane field, clay loam, Oklahoma18	.136	.26	40.0	126.	240.	200.	114.	6.
67 Mixed orchard, silt loam, California	1.80	1.800	74.00	120.0	140.	200.	80.	60.	700.
68 Plum orchard, sandy loam, California26	.140	74.00	7.4	40.	50.	42.6	10.	8.
69 Virgin soil, clay, California	Trace	.100	.88	2.0	30.	480.	478.	450.	8.
70 Small grain, black loam, California	1.40	.160	.04	5.0	34.	640.	635.	606.	10.
71 Raw cactus land, sand and gravel, Calif.	Trace	.120	.32	0.0	12.	0.	0.	—12.	6.
72 Vineyard, sandy loam, California	0.0	.120	.10	7.4	70.	320.	312.6	250.	8.
73 Sugar beets, sandy loam, Kansas	1.20	.260	.12	26.0	160.	700.	674.	540.	2,000.

Nos. 67 and 68 each produced 74 p. p. m. of nitrite nitrogen and Nos. 62, 63, and 65 gave 30, 26 and 30 p. p. m. respectively. In the writer's opinion, these are to be regarded as an indication of interrupted and retarded nitrification rather than of reduction.

The maximum net gain made by any soil in this series was 606 p. p. m. of nitric nitrogen by No. 70, the California black adobe; the average for all positively reacting samples was 214.76 p. p. m. and the minimum 5 p. p. m. by No. 53.

SERIES VII. DRIED BLOOD

Dried blood was employed as the nitrifiable substance in Series VII. Here also we have an increase in the number of soils yielding comparatively large gains; it will be remembered that the first series gave three such and the next, six, while the present one contains eight. Evidently the nitrogen of the dried blood responded more readily to the

TABLE NO. 7.—Nitrification of Dried Blood by Foreign Soils. Nitrogen in Parts Per Million of Air-dried Soil as Nitrites and Nitrates from 100 m.g. Nitrogen as Dried Blood. Duration of Experiment: 6 Weeks at 28 Degrees C.

Number of Sample and Description	Nitrogen as nitrites in parts per million			Nitrogen as nitrates in parts per million					Chlorine in p. p. m.
	Check at begin.	Check at end	Dried blood added	Check at begin.	Check at end	Dried blood added	Total gain	Net gain	
52 Corn field, clay loam, Ohio002	.176	.04	7.0	60.	480.	473.	420.	8.
53 Pine forest, gravelly loam, Georgia.....	Trace	.220	.14	5.0	Trace	6.	1.	6.	78.
54 Apple orchard, loam, Washington04	.340	.80	5.0	90.	210.	205.	120.	40.
55 Apple orchard, heavy clay, Virginia	Trace	.160	.056	9.0	70.	720.	711.	650.	74.
56 Cotton field, sandy loam, Oklahoma ..	.18	.168	.12	11.0	50.	80.	69.	30.	8.
57 Water melons, sandy loam, North Carolina	Trace	.168	.12	7.0	46.	Trace	—7.	—46.	10.
58 Cotton field, sandy loam, Texas12	.160	.06	7.0	50.	200.	193.	150.	98.
59 Cotton field, clay loam, Georgia	Trace	.152	.12	14.0	60.	240.	226.	180.	106.
60 Corn field, clay, North Carolina16	.112	.072	6.0	70.	560.	554.	490.	8.
61 Tobacco field, sandy loam, N. Carolina....	Trace	.100	.18	3.0	20.	0.	—3.	—20.	6.
62 Vineyard, clay loam, California	Trace	Trace	Trace	4.0	24.	180.	176.	156.	26.
63 Alfalfa field, sandy loam, California ..	.04	.100	5.00	4.0	46.	20.	16.	—26.	8.
64 Small grain, sandy loam, California ..	.88	.320	.32	6.0	70.	640.	534.	570.	8.
65 Cotton field, sandy loam, Arkansas60	.132	30.00	4.0	24.	110.	106.	86.	62.
66 Cane field, clay loam, Oklahoma18	.136	.40	40.0	126.	180.	140.	54.	6.
67 Mixed orchard, silt loam, California ..	1.80	1.800	44.00	120.0	140.	220.	100.	80.	700.
68 Plum orchard, sandy loam, California....	.26	.140	70.00	7.4	40.	80.	72.6	40.	8.
69 Virgin soil, clay, Cali- fornia	Trace	.100	.60	2.0	30.	280.	278.	250.	8.
70 Small grain, black adobe, California	1.40	.160	.20	5.0	34.	640.	635.	606.	10.
71 Raw cactus land, sand and gravel. Calif.	Trace	.120	.32	0.0	12.	10.	10.	—2.	6.
73 Vineyard, sandy loam, California	0.00	.120	.52	7.4	70.	400.	392.6	330.	8.
74 Sugar beets, sandy loam, Kansas	1.20	.260	.96	26.0	160.	760.	734.	600.	2,000.

nitrifying agents than did the nitrogen of the ammonia salts; whether this was due to its being present in a more available form or whether the additional food material in the blood stimulated microbic activity can only be conjectured.

Of the twenty-two soils under examination, eighteen or 81.82 per cent. gave gains in nitric nitrogen from the dried blood; four or 18.18 per cent. produced less than the controls.

The eight samples which made the largest gains were Nos. 52, 55, 60, 64, 69, 70, 73, and 74; four came from California, one from Kansas, one from North Carolina, one from Virginia, and one from Ohio; five of these are the same that gave the high yields in the preceding series, while Nos. 52 and 64 are new ones to the list.

Soil No. 60 presents a very interesting case in its behavior toward the different forms of ammoniacal and proteid nitrogen. With the sulphate it gave less nitric nitrogen than the control; when associated with the carbonate, a gain of 290 p. p. m. over the check resulted; and from the dried blood, it produced 490 p. p. m., a very nice illustration of the relative availability of the different nitrogenous compounds for the nitrifying flora of a given soil. Essentially the same relations are brought out in No. 55.

Nos. 57, 61, 63 and 71 all produced less nitrate nitrogen than their controls; these behaved similarly in the preceding series. Nos. 57 and 61 show evidence of some reduction. With Nos. 63 and 71, the negative results appear to be due to retarded nitrification rather than denitrification since some gain has been made over the initial nitrogen of the control but not as much as in the control at the end of the experimental period.

Nos. 67 and 68 gave high nitrites here as in the preceding series and what was said there in this connection will apply equally to the present results.

The maximum gain in nitric nitrogen from the dried blood was made by No. 55 with 650 p. p. m.; the average for the positively reacting soils was 267.39 p. p. m. and the minimum gain 6 p. p. m. by No. 53.

SUMMARY OF FOREIGN SOILS

Seventeen or 77.27 per cent. of the soils under study were able to convert the nitrogen of ammonium sulphate into nitrate nitrogen; the same per cent. accomplished this transformation with ammonium carbonate and eighteen or 81.82 per cent. with dried blood.

The largest total amount of nitric nitrogen was produced from the dried blood; the next largest from the ammonium carbonate, and least from the ammonium sulphate.

The largest amount of nitric nitrogen produced by any one soil from ammonium sulphate was 640 p. p. m. by No. 74, a sandy loam from Kansas; from ammonium carbonate, it was 606 p. p. m. by No. 70, California black adobe; from dried blood, it was 650 p. p. m. by No. 55, a heavy clay from Virginia. It is a significant point that the highest yields from both of the salts were obtained by western soils, while the best return from the dried blood was secured by a southeastern sample. Without exception all of the soils from the southeastern part of the United States made their largest gains from the dried blood; those from Washington, Oklahoma and Texas gave the best results with ammonium carbonate; Arkansas and Kansas (also Colorado)

with ammonium sulphate, while the California samples were divided among the three substances. The fact that these soils upon the basis of their ability to produce nitric nitrogen from different nitrifiable materials fall into more or less well defined natural geographical groups, suggests rather strongly the presence of a nitrifying flora in each group, distinct to a greater or less degree from that of another group. Further evidence in support of this view is to be had in the sequence in which the different nitrifiable substances yielded nitric nitrogen in the different groups. All of the southeastern samples, that reacted positively, responded in the following order: Dried blood, ammonium carbonate, ammonium sulphate. The Texas-Oklahoma group gave this sequence: Ammonium carbonate, dried blood, ammonium sulphate. The Colorado samples constitute a third class, in which the prevailing order is ammonium sulphate, ammonium carbonate, dried blood. The number of samples in the first two groups is too small to warrant any positive assertions along this line, but the results are certainly suggestive.

Seven of the nine or 77.77 per cent. of the soils that made the largest gain in nitric nitrogen from dried blood gave second place to ammonium carbonate and third place to ammonium sulphate; seven out of seven or 100 per cent. of those that produced most from ammonium carbonate followed next with the dried blood and then the ammonium sulphate; three of the five or 60 per cent. of those that gave first place to ammonium sulphate, followed with dried blood and then ammonium carbonate.

The average amount of nitric nitrogen produced by a positively reacting soil in each series was as follows: Ammonium sulphate, 152.82 p. p. m.; ammonium carbonate, 214.76 p. p. m.; dried blood, 267.39 p. p. m.; check 47.08 p. p. m.

The average amount of nitric nitrogen produced by a foreign soil in each series, all samples taken into consideration, positive and negative, was as follows:

Ammonium sulphate, 110.36 p. p. m.; ammonium carbonate, 161.63 p. p. m.; dried blood, 214.72 p. p. m.; check 44.93 p. p. m.

Considering the positively reacting soils in each series or those which showed an increase in nitric nitrogen over the checks, we find that 29.41 per cent. made their largest gains from ammonium sulphate, 41.48 per cent. from the ammonium carbonate; 50 per cent. from the dried blood; 29.41 per cent. made their second highest gains from ammonium sulphate; 35.29 per cent. from ammonium carbonate and 44.44 per cent. from the dried blood. 41.18 per cent. produced their third largest yields from the ammonium sulphate; 22.22 per cent. from the ammonium carbonate and 5.56 per cent. from the dried blood. The results point clearly to dried blood as the most fertile source of nitrogen for the formation of nitrates by the majority of the foreign soils; ammonium carbonate comes second and ammonium sulphate last.

22.73 per cent. of the soils produced less nitric nitrogen in the presence of the ammonium sulphate than the controls; 22.73 per cent. gave less with the ammonium carbonate than the checks, and 18.18 per cent. yielded less from the dried blood than the controls.

COMPARATIVE SUMMARY OF COLORADO AND FOREIGN SOILS.

A comparison of the nitrifying efficiencies of these two groups of soils, taken as integrals, shows at a glance that the Colorado samples are superior to the others in their power of converting ammoniacal and proteid nitrogen into nitric nitrogen.

Considering only those soils that gave a positive reaction for nitrification, we find that the average net gain in nitric nitrogen made by a Colorado soil from ammonium sulphate was 672 p. p. m., while that of a foreign sample amounted to only 150.82 p. p. m.; again, the former

TABLE NO. 8.—Increase in Nitric Nitrogen in Colorado Soils and Foreign Soils Without the Addition of Any Nitrifiable Substance. Nitrogen in Parts Per Million of Air-dried Soil. Duration of Experiment: 6 Weeks at 28 Degrees C.

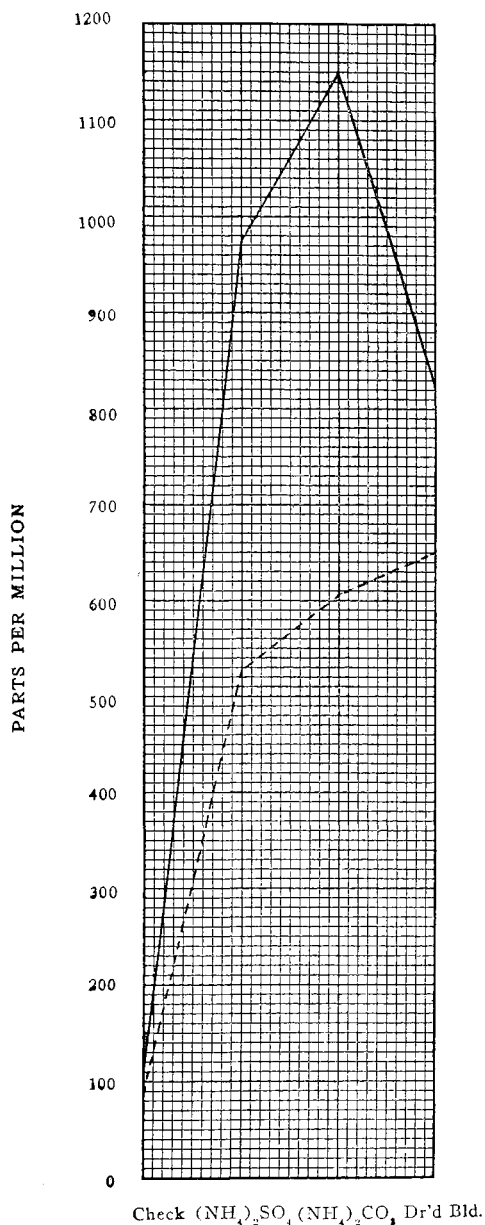
Colorado Soils				Foreign Soils			
Sample No.	At beginning	At end	Net gain	Sample No.	At beginning	At end	Net gain
75	7.	64.	57.	52	7.	60.	53.
76	54.	110.	56.	53	5.	Trace	—5.
77	26.	76.	50.	54	5.	90.	85.
78	140.	140.	0.	55	9.	70.	61.
79	5.	66.	61.	56	11.	50.	39.
80	13.	50.	37.	57	7.	46.	39.
81	1360.	1360.	0.	58	7.	50.	43.
82	46.	120.	74.	59	14.	60.	46.
83	6.	100.	94.	60	6.	70.	64.
84.	Trace	68.	68.—	61	3.	20.	17.
85	6.	60.	54.	62	4.	24.	20.
86	130.	240.	110.	63	4.	46.	42.
87	6.	40.	34.	64	6.	70.	64.
88	70.	100.	30.	65	4.	24.	20.
89	180.	260.	80.	66	40.	126.	86.
90	13.	60.	47.	67	120.	140.	20.
91	Trace	40.	40.—	68	7.4	40.	32.6
92	320.	320.	0.	69	2.	30.	28.
93	4.	48.	44.	70	5.	34.	29.
94	34.	92.	58.	71	0.	12.	12.
95	600.	640.	40.	73	7.4	70.	62.6
96	20.	60.	40.	74	26.	160.	134.
97	20.	132.	112.				

yielded 520.88 p. p. m. from ammonium carbonate and the latter but 214.76 p. p. m.; from dried blood the Colorado soils averaged 431.50 p. p. m. as compared with 267.39 for the other series. This means that the Colorado samples produced an average of 521.18 p. p. m. or 345.56 percent. more nitric nitrogen from ammonium sulphate than the other soils; that they yielded an average of 306.12 p. p. m. or 142.54 per cent. more from the ammonium carbonate, and 164.11 p. p. m. or 61.37 per cent. more from the dried blood. (See Plate II.)

The maximum net gain produced by a Colorado soil from ammonium sulphate amounted to 972 p. p. m., while the highest foreign (1)

(1) Inasmuch as the Kansas soil is in all essentials a Colorado soil, it has not been considered in this comparison.

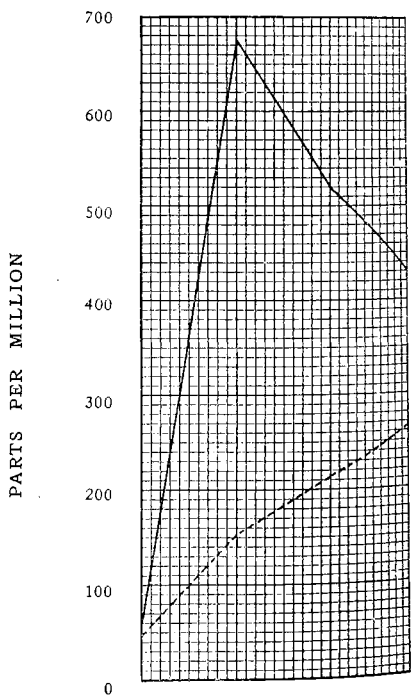
PLATE I.—MAXIMUM AMOUNTS OF NITRIC NITROGEN PRODUCED FROM THE DIFFERENT NITRIFIABLE SUBSTANCES



Check $(\text{NH}_4)_2\text{SO}_4$ $(\text{NH}_4)_2\text{CO}_3$ Dr'd Bld.

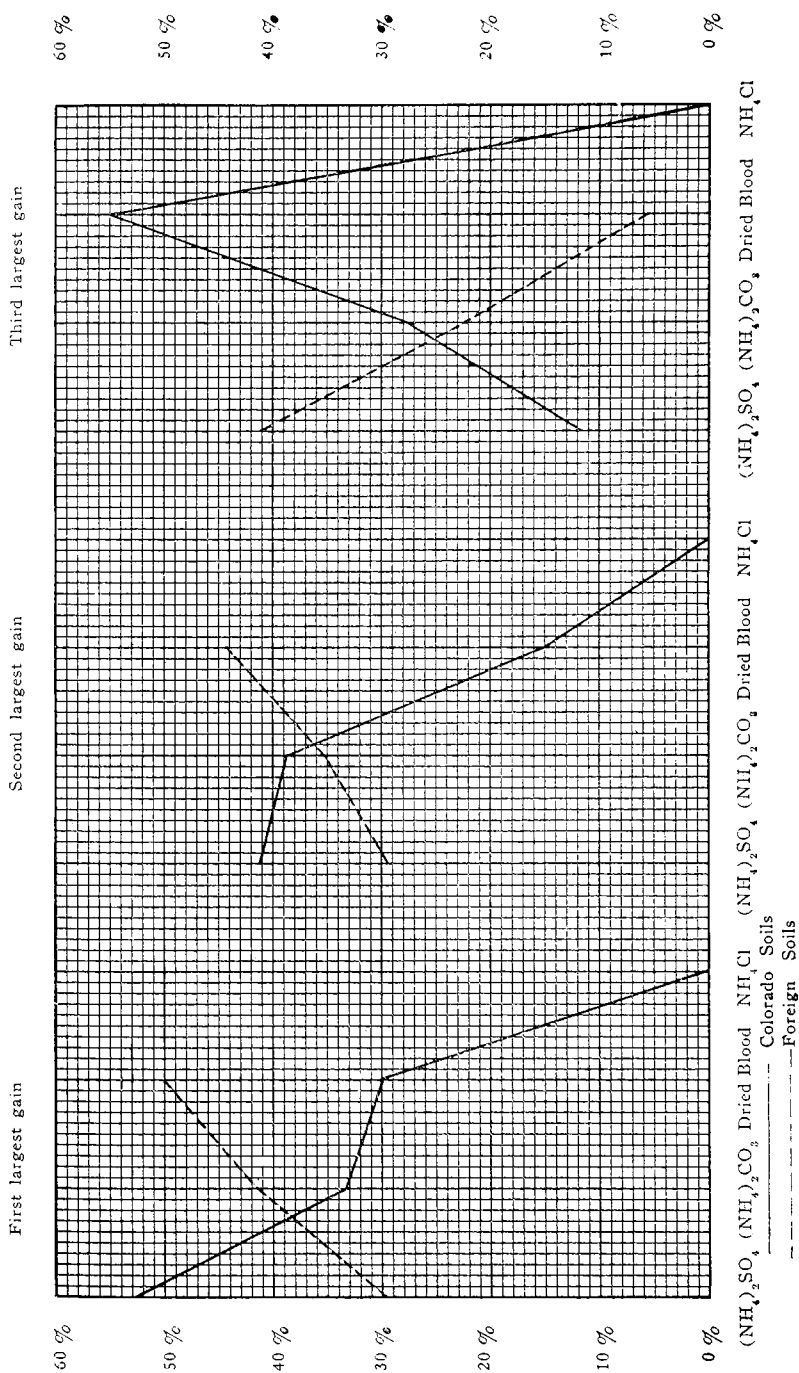
———— Colorado Soils
 - - - - - Foreign Soils

PLATE II.—AVERAGE AMOUNT OF NITRIC NITROGEN PRODUCED FROM THE DIFFERENT NITRIFIABLE SUBSTANCES.



Check $(\text{NH}_4)_2\text{SO}_4$ $(\text{NH}_4)_2\text{CO}_3$ Dr'd Bld.

PLATE III. GRAPHIC REPRESENTATION OF THE PERCENTAGE OF POSITIVELY REACTING COLORADO AND FOREIGN SOILS THAT MADE THEIR FIRST, SECOND AND THIRD LARGEST GAINS IN NITRIC NITROGEN FROM THE DIFFERENT NITRIFIABLE SUBSTANCES.



yield was 526 p. p. m.; from ammonium carbonate, the largest net gain by a Colorado sample was 1148 p. p. m., by a foreign soil, 606 p. p. m.; from dried blood the former gave 820 p. p. m., the latter 650 p. p. m. (See Plate I.)

In looking over the net gain columns of the Colorado tables one sees but few positive results under 300 p. p. m. and many above 500 p. p. m. with the exception of the ammonium chlorid series. On the other hand, an inspection of the corresponding columns of the foreign group shows many figures between 20 and 90 p. p. m. and only a few above 250 p. p. m.

A comparison of the average gains in the two sets is given in Tables 9 and 10.

The second conspicuous difference between the two series we find in their inverse ability to nitrify the different materials placed at their disposal. To illustrate—52.94 per cent. of the Colorado samples made their largest net gains in nitric nitrogen from ammonium sulphate as compared with 29.41 per cent. of the others; 33.33 per cent of the soils

TABLE NO. 9.—Average Net Gain in Nitric Nitrogen by Positively Reacting Colorado and Foreign Soils in Parts Per Million of Air-dried Soil.

Nitrifiable substance added:	Nothing	$(\text{NH}_4)_2\text{SO}_4$	$(\text{NH}_4)_2\text{CO}_3$	Dried Blood
Colorado Soils.....	59.30	672.00	520.88	431.50
Foreign Soils.....	47.08	150.82	214.76	267.39
Excess of Colorado Soils over Foreign Soils.....	12.22	521.18	306.12	164.11
Gain per cent. by Colorado Soils	25.95	345.56	142.54	61.37

TABLE NO. 10.—Average Net Gain in Nitric Nitrogen by Colorado and Foreign Soils in Parts Per Million of Air-dried Soil, all Samples Considered.

Nitrifiable substance added:	Nothing	$(\text{NH}_4)_2\text{SO}_4$	$(\text{NH}_4)_2\text{CO}_3$	Dried blood	NH_4Cl
Colorado Soils.....	51.30	484.26	398.34	341.30	56.86
Foreign Soils.....	44.93	110.36	161.63	214.72	
Excess of Colorado Soils over Foreign Soils.....	6.37	373.90	236.71	126.58	
Gain per cent. by Colorado Soils	14.17	338.80	146.45	58.95	

from Colorado produced their highest yields from ammonium carbonate as against 35.29 per cent of the others; 30 per cent. of those from Colorado made their maximum gains with dried blood as compared with 44 per cent. of those from outside of the state. From this, it is very clear that the majority of the Colorado soils nitrify ammonium sulphate most readily and dried blood least easily, whereas exactly the opposite is true of the foreign samples—ammonium sulphate least easily and dried blood most readily. (See Plate III.)

The superior nitrifying efficiency of the Colorado soils and the inverse relation of their nitrifying power to that of the foreign samples suggest the following:

1. The conditions for nitrification in Colorado soils are superior to those found in the other soils.

2. Colorado soils possess a vigorous nitrifying flora.
3. The nitrifying flora of the Colorado soils is distinct from that found elsewhere; it is either made up of entirely different organisms, or, if the same organisms, they have become so changed by their environment that they behave like different strains.

CONCLUSIONS

Many cultivated soils of Colorado contain a vigorous nitrifying flora capable of transforming ammoniacal nitrogen into nitrate nitrogen.

Both our normal soils and those in the incipient stage of the niter trouble possess this power in a very marked degree.

Compared with soils from twenty-two other localities outside of the state, the Colorado soils examined are very superior in nitrifying efficiency.

The nitrifying efficiency of Colorado soils bears an inverse relation to that of the foreign soils when referred to ammonium sulphate, ammonium carbonate and dried blood as the nitrifiable substances.

Colorado soils produced their highest average gains in nitric nitrogen from $(\text{NH}_4)_2\text{SO}_4$; the next largest from $(\text{NH}_4)_2\text{CO}_3$ and the lowest from dried blood. The foreign soils produced their largest average yields in exactly the reverse order.

The nitrifying flora of the Colorado soils is distinct from that found in the majority of the foreign samples; it is either made up of entirely different organisms, or, if the same organisms, they behave like different strains.

Excessive nitrates do not appear to interfere seriously with nitrification provided the chlorin is low.

Excessive chlorin, with or without excessive nitrates, inhibits nitrification.

Active nitrification takes place in the brown crust from the niter spots provided the chlorin is not excessive.

The sample of raw adobe clay examined was deficient in nitrifying efficiency.

The results of this study together with those of our two previous investigations justify the position that the excessive nitrates present in certain Colorado soils have resulted from the combined action of nitrogen-fixing, ammonifying and nitrifying organisms.

ACKNOWLEDGMENTS

I beg to acknowledge my indebtedness to the following persons for their courtesy in sending me samples of soil from localities outside of Colorado: Professor Hugh G. Faust, Miss Ida Wray Ferguson, Dr. E. B. Fred, Mr. C. C. Hommon, Dr. J. V. Knapp, Professor F. B. Paddock, Mr. Joe F. Pool, Professor E. S. Porter, Mrs. S. Ranne, Mr. G. C. Ranne, Mr. W. C. G. Sackett, Professor G. E. Searcher, Dr. Mary Wetmore, Mr. Charles E. White, Mr. J. Y. Whitaker.