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A BACTERIOLOGICAL METHOD FOR DETERMINING MINERAL SOIL DEFICIENCIES BY USE OF THE SOIL PLAQUE

By Walter G. Sackett and Laura C. Stewart

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Colorado Experiment Station
Fort Collins

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TABLE OF CONTENTS

A BACTERIOLOGICAL METHOD FOR DETERMINING MINERAL SOIL DEFICIENCIES BY THE USE OF THE SOIL PLAQUE

By Walter G. Sackett and Laura C. Stewart

There is probably no phase of soil science that has offered more interesting problems to the investigator or more important, practical difficulties to the husbandman than the maintenance of soil fertility. As long as the land remains productive, it is hard to convince some of our farmers that this condition cannot exist always, and that sooner or later they will be compelled either to adopt measures for conserving the fertility of their fields or to resort to the use of large quantities of commercial fertilizer. The only alternatives that present themselves are unprofitable farming and starvation. The thousands of forlorn and deserted farmsteads thruout our country testify to the truth of this statement.

We no longer have the extensive frontiers of 50 years ago with their countless acres of virgin soil, rich in natural fertility, and the time is past when we can solve our problems of soil depletion simply by moving to a section of new land.

In the arid West, where the rainfall amounts to less than 20 inches, frequently from 7 to 12, the natural fertility has not been removed by leaching to the same degree as in the rainbelt. For years, it has been necessary in the Eastern, Central and Southern states to use large quantities of commercial fertilizer to secure crop yields that are profitable. Until quite recently, Colorado soils have produced extraordinarily well, altho it has been observed that where 10 to 15 tons of barnyard manure per acre were applied, appreciably greater returns were secured. It appears now that we have arrived at a point in our agriculture where we have consumed the reserve of some of the mineral plant foods such as phosphorus and limestone, in an available form, and that our crop demands are exceeding the supply with the result that yields have decreased noticeably in very recent years. Of course, we can continue to cultivate our lands until they reach the same worthless, depleted condition of some New England farms, but it is far better business to maintain the soil in a producing condition when this can be done with little effort and expense than to allow it to depreciate to the point where the cost of restoring it would be prohibitive. Too often the foresight of our American farmers is blinded by the greed for present gains. Too little attention is paid to the ultimate fertility of the soil.

One has only to visit Long Beach or San Diego, California, to find the explanation for many run-down farms. Here and elsewhere we find hundreds of retired farmers from the middle West who accumulated a sufficient surplus from their soil when it was new and productive to provide them with comfortable incomes. They got the juice out of the orange and left the peeling behind. Practically all of these people owned their own places at one time or another, and as long as they cultivated the land themselves, their personal pride and interest in their properties were incentives to keep up the fertility. However, when they moved away from the farms and rented them to irresponsible strangers, it was quite a different story. Not infrequently the owner's share of the proceeds was insufficient to pay the taxes.

We need not argue the tenant question with those of you who have been farmers, for your experience and ours has doubtless been the same. The quickest and surest way to ruin a good farm is to rent it. To be sure there are some good tenants who have the owner's best interest at heart as well as their own, but there are many others who are little more than one stage removed from gypsies, and who concern themselves solely with getting all from the land possible with the least expenditure of time and effort. They give no thought to the future fertility of the soil, realizing that, in all probability, they will move to an equally poor farm the next season. Why worry about fertilizing the land for the next fellow? While the argument may be sound, this practice, continued year after year, has resulted in such soil depletion that thousands of acres of our best land are today either worthless or producing less than 20 percent of a normal yield.

Few of us realize what is happening to the fertility of our fields when we haul off 12 tons of sugar beets or 4 tons of alfalfa per acre every year. Here are a few figures* showing the amounts of the ordinary fertilizers which are removed from the land each season by some of the common farm crops.

Table 1.—Pounds fertilizer removed from soil per acre each year by common farm crops.

Crops	Pounds fertilizer removed per acre each year.		
	Sodium nitrate	Treble Superphosphate	Potassium sulphate
50 bu. wheat, 2½ tons straw	582.46	81.39	129.26
50 bu. corn, 1½ tons stalks	448.98	58.50	79.11
4 tons alfalfa	1,213.47**	91.56	213.94
300 bu. potatoes	382.24	66.13	200.57
12 tons sugar beets	364.03	54.93	209.92
300 bu. apples, leaves and twig growth	679.54	71.22	242.92

*Adapted from Hopkins' "Soil Fertility and Permanent Agriculture," page 154.
**Part of this comes from the air.

The table is self-explanatory, but let us follow thru sugar beets as an illustration. You will find sugar beets in the first column under "Crops;" in the next column is given the number of pounds of nitrogen in the form of sodium nitrate, that is 364.03, which are taken from each acre of land each year by a 12-ton crop of beets; in the third column is found the number of pounds of phosphorus in the form of treble superphosphate, that is, 54.93; in the last column is given the number of pounds of potassium in the form of potassium sulphate, that is, 209.92 pounds. In other words, if you are going to maintain the fertility of your land undiminished, these are the amounts of the different fertilizers it will be necessary to add per acre each year. A portion of this will be restored thru natural biological processes in the soil, but a great deal of it will have to be supplied from outside sources, either as barnyard manure or as commercial fertilizers.

Compared with sugar beets, it is interesting to note that a 4-ton crop of alfalfa removes twice as much phosphorus as the beets. In view of this, it is not surprising that our alfalfa tonnage has been dropping off, and that our alfalfa lands are showing a phosphorus deficiency, after growing this crop for 25 to 30 years.

The statement is sometimes made by men who pride themselves on being good farmers that everything that is raised on the place is fed there, and that the manure is put back on the land, the inference being that because of this practice, which is very commendable, the soil is losing nothing. If anyone really believes this, let him not deceive himself longer, for the fertility of the farm is walking away, literally, in the livestock that is sold.

If farming is to be carried on profitably, with the prevailing low prices of produce, we must find a way of raising more tons per acre with a minimum additional cost of production. Improved agricultural practice, better systems of rotation and increased soil fertility suggest themselves as possible means to this end. Of these three, increasing the soil fertility offers one of the most certain ways of obtaining larger yields.

In the pages which follow, we hope to interest you in a method for determining your soil needs so that you can apply fertilizer intelligently when and where necessary, and conserve it where it is not required.

METHODS OF TESTING SOIL FOR MINERAL DEFICIENCIES

CHEMICAL ANALYSIS

The belief is rather general among farmers that a chemical analysis will show what a soil needs to make it more productive. Were it possible to extract from the soil by chemical means exactly what a

plant can obtain, this would be possible, but the chemist, in his efforts to imitate the action of plant roots in dissolving the mineral particles, employs weak acids as solvents. While this approaches natural processes, in a measure, it gives only an approximate idea of the amount of material which is in a form that plants can utilize, since the mineral food which a growing crop can take from the soil and that which is yielded to chemical methods may be quite different. From this, it is evident that a chemical analysis is of limited value except where a marked excess or deficiency exists.

It is not our intention to minimize or disparage in the least either the importance or the value of a chemical analysis or test, but we do question whether it is giving us the real information we are seeking, and whether we cannot secure more exact data bearing upon actual plant requirements by a different method of approach.

EXPERIMENTAL PLOTS

One way of accomplishing this is by applying different fertilizers at varying rates to experimental plots which are then planted to growing crops. At the end of the season, yields are taken and conclusions are drawn from the results as to the relative benefits of the several treatments. This method has been used very extensively by the experiment stations in the United States and abroad. It is so limited in its general application, however, that it possesses little value for the individual farmer.

To illustrate this point: Let us assume that there are two fields on opposite sides of a road; they are identical with respect to type, texture, drainage, slope and exposure. The one on the east side has been manured and rotated systematically; it produces well and shows no deficiencies. The one on the west has been cropped for 30 years continuously by roving tenants who had no thought of maintaining or building up its fertility; the crops here are almost a total failure for the soil is deficient in everything. Obviously the results obtained from experimental fertilizer plots on these two tracts would be very different and would not apply to each other. The necessity of determining the fertilizer needs of each farm separately is apparent from this illustration, for the capacity of a soil to yield profitable returns depends, in most cases, more upon the way the land has been handled and taken care of than upon the type.

NEUBAUER TEST

While experimental plots on every farm might be an ideal arrangement, they would be impracticable for several reasons. An approach to this has been made, however, by the Neubauer(3) method of testing for deficiencies in phosphate and potash. Here, rye plants are grown in the laboratory for 14 days upon the soil under examina-

tion, and from a chemical analysis of the plants the shortage of phosphate and potash is determined. The results obtained by this method appear to be quite dependable, but if thousands of samples have to be examined in a limited time, as would be the case if every farmer were to have his soils tested before spring planting, this procedure would not be feasible because of the time and expense involved. What we need is a simple, rapid, sensitive, inexpensive biological method for determining whether there is enough available mineral plant food in the soil to grow crops satisfactorily.

BACTERIOLOGICAL SOIL PLAQUE TEST

Winogradsky and Ziemiecka (6) in their studies upon the nitrogen fixing power of soils observed that the bacterial genus, Azotobacter, would produce characteristic colonies spontaneously upon a soil plaque, provided that the soil contained suitable energy material together with certain limiting mineral elements, namely, phosphate, potash and calcium carbonate; further that the absence of any one of these might account for the failure of the nitrogen-fixing organism to develop. While this investigation was conducted primarily to determine the reason for the variation in the nitrogen-fixing power of different soils, Winogradsky states:

"The idea of combining the test of the spontaneous culture with the research of the limiting mineral factor is fully indicated. It suffices for this end to add to the soil calcium carbonate or soluble phosphate; if these are the salts that are lacking in the fertility, one will see the fertilized portion produce notable growths of Azotobacter, while the portions not treated, or wrongly, remain barren."

And further:

"It is clear, however, that the reaction of these microbes, so sensitive to limiting mineral factors, can serve to indicate these latter in the soil and that with a sensitiveness very superior to chemical methods."

In principle, the soil plaque method is simply this:

The mineral food requirements of Azotobacter and farm crops being so similar, we can use the development of Azotobacter colonies (See Fig. 1) as an indication of what might reasonably be expected to take place if the particular soil were planted to sugar beets, corn or some other crop. By adding different mineral fertilizers to the plaques, either singly or in combination, and by observing, 72 hours later, both the number and luxuriance of the Azotobacter colonies, which appear either spontaneously or as the result of inoculation, in comparison with those which are present on the untreated plaque, we can gain a fairly accurate idea of both what and how much the soil lacks.

The great advantage which this method possesses over both chemical and other biological procedures is the short time that is required to complete the test. A crop of mature Azotobacter colonies, easily visible to the naked eye, can be obtained in 72 hours, whereas, with the Neubauer test, results can not be expected in less than 20 to 30 days, and, with the field plot, a growing season is necessary.

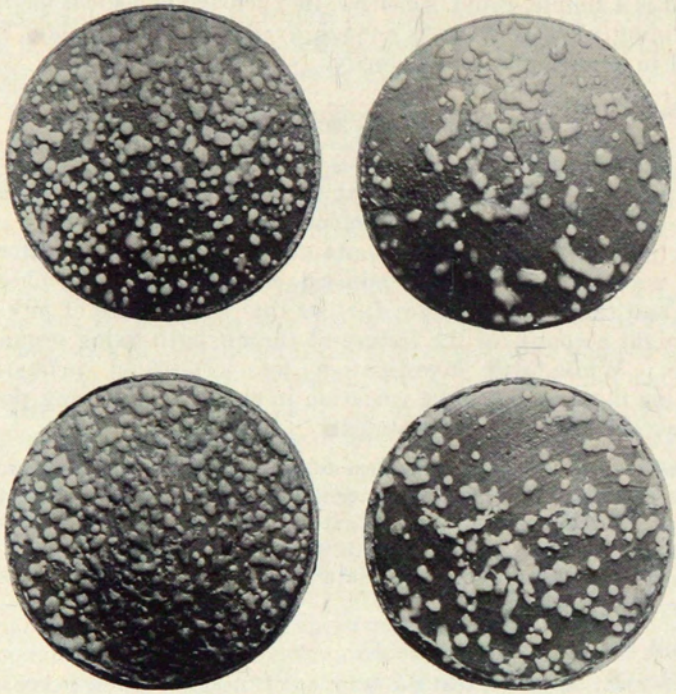


Fig. 1.—Azotobacter colonies on soil plaques.

DESCRIPTION OF THE METHOD

Collection of Samples

It is of utmost importance that the sample be representative of the entire area. In order to obtain such a sample it is our practice to take a number of small samples over the field at intervals of 50 paces. Either a soil auger or shovel can be used for this purpose. In case the latter is employed, as is our custom, it is thrust into the ground to a depth of approximately 6 inches, the first shovelful of soil is thrown out, a thin slice, one-half inch thick is shaved from the back side of the hole and placed in a pail or sack. Sampling in this manner is continued across the tract until 25 to 50 portions have been obtained. These are all emptied from the pail upon a large piece of canvas and mixed thoroly. A sample of not less than 3 pounds, or what will fill a 1-pound coffee can, is withdrawn from this composite

for the test. Such a sample should be taken from each field on which information is desired.

LABORATORY PROCEDURE

On arriving at the bacteriological laboratory, the soils are spread out upon large sheets of paper and air dried which is usually accomplished over night in our arid climate. They are next pulverized to pass a 20-mesh sieve and stored in friction-top tin cans until we are ready to make the plaques. Samples have been kept in a dry condition in this manner for more than 2 years without any apparent loss in the vigor of the Azotobacter cells.

SOIL REACTION.—The hydrogen ion concentration of the soil is determined next colorimetrically (5) and if the pH is less than 6.8, 5 grams of precipitated calcium carbonate are added to each 50 grams of soil when the plaques are made. This provides a large excess, but no deleterious effect has been observed from using as much as 33 percent.

Gainey (1) has shown recently that the addition of 5 percent of calcium carbonate to an acid soil with an initial pH of 4.0 gave a pH of 7.1 after 84 days; 0.6 percent was necessary to raise the pH to 6.0 which he regards as the "maximum hydrogen ion concentration in the soil solution compatible with the existence therein of an active Azotobacter flora."

By using large quantities of calcium carbonate, as is our practice, neutralization of the acid is brought about in a shorter length of time, and more favorable conditions for growth are established earlier than when less is employed.

While there may be some development of Azotobacter colonies on plaques with a pH between 6.0 and 6.8, the addition of calcium carbonate insures a rapid and vigorous growth in soils that otherwise might have produced either no colonies or only very feeble ones.

Fig. 2.—Soil deficient in calcium carbonate and phosphate; inoculated. Reading left to right: Phosphate added; phosphate and calcium carbonate added.

In Fig. 2 is illustrated a set of plaques made from an acid soil deficient in phosphate. The plaque on the left received phosphate,

but no calcium carbonate; the one on the right was given phosphate and 10 percent of calcium carbonate. The first produced no Azotobacter colonies, while the latter gave a luxuriant growth of the same. In this case, calcium carbonate was clearly necessary for the development of Azotobacter.

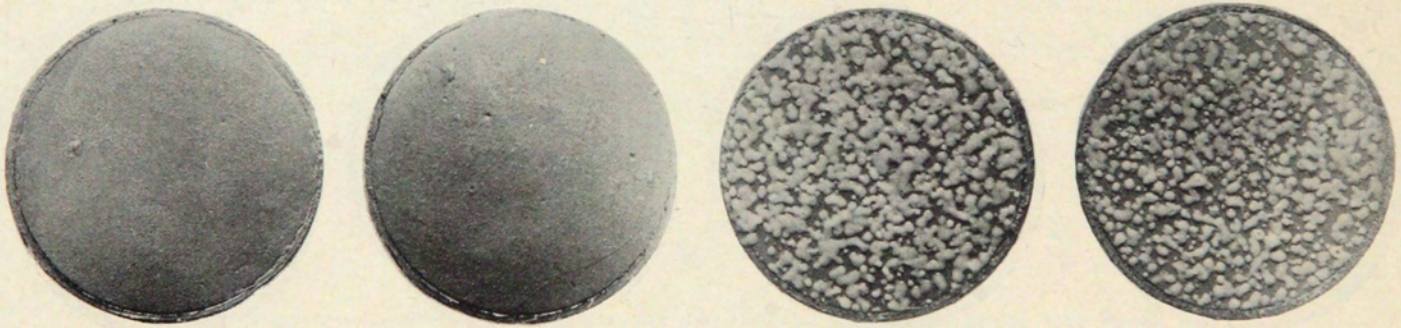
MAKING OF PLAQUES.—For each test, four 50-gram portions of the sample are weighed out; 2.5 grams of Kingsford's cornstarch are added to each as energy material and mixed thoroly with the dry soil. The mixing is done in the half of a glass culture dish (90 x 45 m.m.); a heavy glass tumbler or beaker serves equally well. To portion No. 1, which is to be the check, we add distilled water from a graduated pipette, little at a time, with constant stirring until the plastic mass has the consistency of putty or modeling clay used in kindergartens, possibly a little more moist. The exact amount of water used in this operation must be noted and recorded since all plaques must have the same moisture content. This wet soil or mud, if you please, is now transferred to the half of a small glass culture dish, like a petri dish (50 x 20 m.m.), packed in carefully to make a mud pie or plaque, the top being left slightly convex, and the surface smoothed to a nice polish with a glass microscope slide moistened with distilled water. The finished plaque is placed at once in a large covered culture dish (24 cm. in diameter) on moist blotting paper to prevent it from drying out while the remaining three plaques of the set are being made. A piece of blotting paper should be fitted into the top of the cover to absorb any water of condensation, otherwise this may drop upon the surface of the plaques and spoil the development of characteristic colonies.

To portion No. 2 are added 5 c.c. of a 3 percent solution of potassium sulphate (K_2SO_4), equivalent to 0.15 grams, and enough distilled water to equal, with the 5 c.c. of potassium sulphate solution, the total amount of liquid used in making the check plaque. For example, if the check required 15 c.c. of distilled water, we would use 5 c.c. of the potassium sulphate solution and 10 c.c. of distilled water. This is mixed thoroly and moulded into a plaque for potash deficiency.

The third portion receives 5 c.c. of a 6 percent solution of disodium phosphate ($Na_2HPO_4 . 12 H_2O$) equivalent to 0.3 grams and distilled water as above; it is mixed and made into a plaque for phosphate deficiency. Occasionally, with some soils, a little less water is required to give this plaque the proper consistency so the water should be added cautiously to avoid getting the plaque too wet.

The fourth portion is given 5 c.c. of a 3 percent solution of dipotassium phosphate (K_2HPO_4) equivalent to 0.15 grams and distilled water; it is mixed and fashioned into a plaque for both phosphate and potash deficiencies.

Sample No. 7



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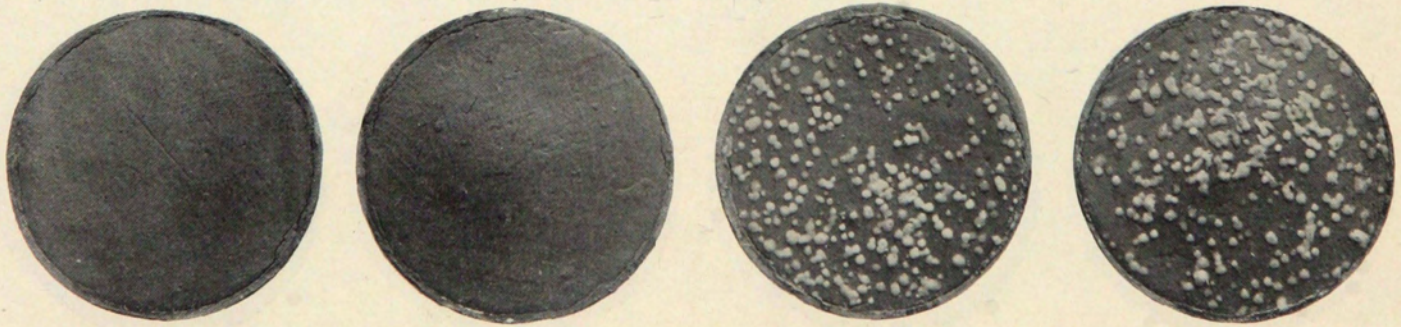
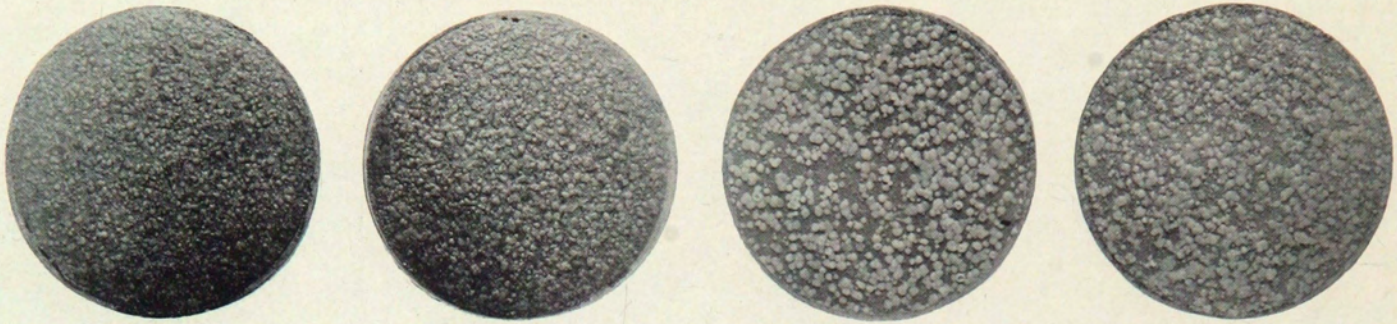


Fig. 3.—Soils very deficient in phosphate, not deficie nt in potash. Reading left to right: Check, nothing added; potash added; phosphate ad ded; phosphate and potash added.

Sample No. 99



Sample No. 51

Fig. 4.—Soils not deficient in either phosphate or potash. Reading left to right: Check, nothing added; potash added; phosphate added; phosphate and potash added.

Sample No. 144

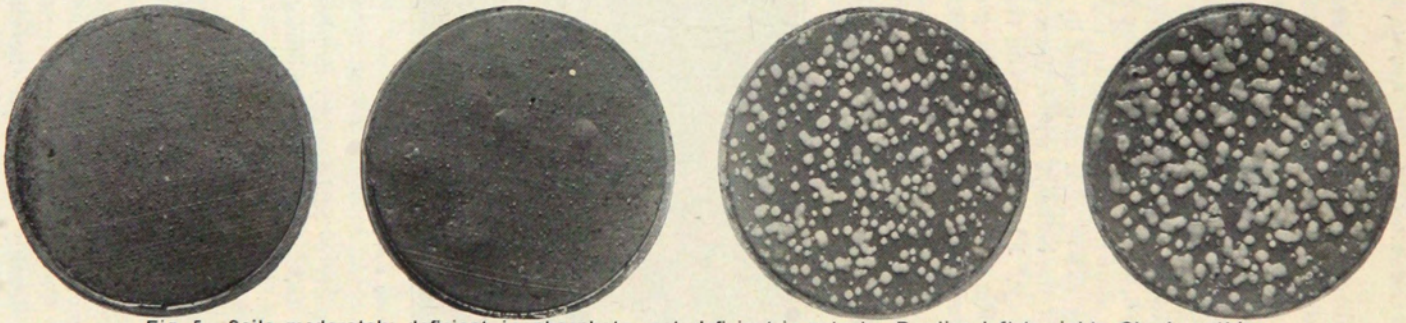


Fig. 5.—Soils moderately deficient in phosphate, not deficient in potash. Reading left to right: Check, nothing added; potash added; phosphate added; phosphate and potash added.

Sample No. 1053

Fig. 6.—Soils slightly deficient in phosphate, not deficient in potash. Reading left to right: Check, nothing added; potash added; phosphate added; phosphate and potash added.

The four plaques, prepared as described, are placed in the large culture dish mentioned, and are incubated for 72 hours at 30°C. At the end of this period, if the correct limiting factors or deficiencies have been supplied, very characteristic Azotobacter growths will have appeared on the surface of the plaques as starchy, waxy white, raised, moist, glistening, circular colonies, easily visible to the naked eye. Where the deficiency has not been met, the plaques remain bare; and if there is no deficiency, all plaques exhibit equally good growth. Photographs of such typical plaques are shown natural size in Figs. 3 and 4. Where only a partial deficiency exists, it is indicated by a reduction in the vigor, size and number of the colonies which develop, depending upon the degree of the deficiency. Fig. 5 shows a soil that is moderately deficient in phosphate and Fig. 6 one that is only slightly so. This phase of the work is treated more fully under "Classification of Deficiencies."

The equipment used in making the tests is illustrated in Fig. 7. Aluminum dishes for making the plaques, altho more expensive than

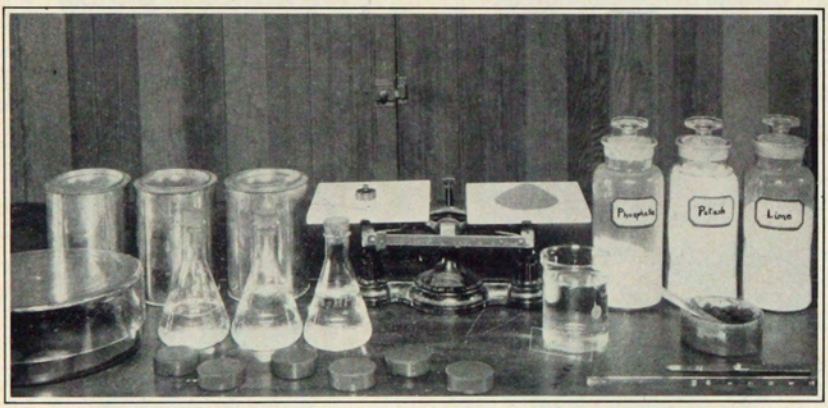


Fig. 7.—Equipment used in making soil plaques.

glass, are obtainable, and appear to be equally as satisfactory as glass. In our laboratory we use culture dish No. 3500, 50 x 20 m.m., obtained from Greiner and Friedrichs, Stutzerbach i/Th., Germany.

In arriving at the amount of the different mineral fertilizers to add to each plaque, we first prepared a series of 20 plaques with $Na_2HPO_4 \cdot 12H_2O$, varying the rate from .0014 gram to 0.67 gram with 50 grams of soil. These are equivalent to 50 and 24,000 pounds of treble superphosphate per acre foot, respectively. The smallest amount which gave the optimum colony development on an average deficient soil was selected as the standard dosage. This proved to be 0.3 gram $Na_2HPO_4 \cdot 12H_2O$ and while this is considerably more than the cus-

tomary rate used in the field, the smaller quantities corresponding to field applications produced only comparatively feeble colonies on deficient soils.

We are endeavoring to standardize the potassium dosage in a manner similar to the phosphate and are using tentatively for each 50 grams of soil a weight of K_2SO_4 containing approximately the same amount of potassium as is present in a weight of K_2HPO_4 equivalent in phosphate to 0.3 grams $Na_2HPO_4 \cdot 12H_2O$. This is 0.15 gram.

For the combination of phosphate and potash we are using for each plaque a weight of K_2HPO_4 equivalent in phosphate to 0.3 gram $Na_2HPO_4 \cdot 12H_2O$, that is, 0.15 gram.

H_3PO_4 SUBSTITUTED FOR Na_2HPO_4 IN VERY BASIC SOILS.—Occasionally we have encountered soils with a basicity of pH 8.4, and in order to avoid increasing this beyond the tolerance of Azotobacter by the addition of a sodium salt for phosphate deficiency, we have used the P_2O_5 equivalent as phosphoric acid, namely 85 mg. per 50 grams of soil. Plaques in which phosphoric acid was substituted for disodium phosphate are shown in Fig. 8.

Fig. 8.—Heavy soil deficient in phosphate; strongly basic; H_3PO_4 substituted for Na_2HPO_4; 20 percent ground quartz added; pH 8.4; inoculated. Reading left to right: Check, nothing added; H_3PO_4 added.

AERATION OF HEAVY SOILS.—We sometimes experience difficulty in securing satisfactory Azotobacter development on extremely finely divided clay, known locally as "adobe." This is the kind of soil that becomes "greasy," "soapy" and very slippery when wet; the soil that puddles badly, bakes and cracks when dry. The conditions for aeration in this type are so poor, due to its physical character, that no Azotobacter appear to be naturally present, and even when inoculated with a pure culture of this organism, often no bacterial growth takes place. With such soils, it is our practice to add 10 grams of ground quartz per plaque and to inoculate. This usually gives positive results. The plaques shown in Fig. 8 received this treatment.

Again there are soils so sandy that it is difficult to obtain a sufficiently smooth surface on the plaque to make the Azotobacter

colonies plainly visible. Under such circumstances, we add 10 to 20 percent of pure kaolin. Such samples usually respond better to sugar than to corn starch as noted below.

ENERGY MATERIAL.—Occasionally we have tested soils which give no Azotobacter colonies with starch even when all the other deficiencies have been supplied. This appears to be due both to the inability of the Azotobacter to utilize the cornstarch as such and to the absence of the anaerobes which are necessary to convert it into compounds available for Azotobacter. If either sucrose or mannite is substituted for the starch in these cases, good growth usually results.

In this connection Winogradsky and Ziemiecka (6) state:

"This modification has shown itself necessary by the fact that the lighter soils, rich in germs, have not given very visible colonies, but as soon as one replaces the starch by mannite, an immense number of colonies have arisen at the end of the period of twenty-four hours. The reason of this difference is probably to be sought in the fact that the clayey soils contain many more anaerobes, which act rapidly upon the starch in the transformation into dextrine, one of the nutrients of preference of Azotobacter (Omeliansky) while in the lighter soils, very poor in anaerobes, these transformations probably proceeded at an insufficient pace for a massive multiplication of these fixers. One will take then one or the other of these substances according to the qualities of the soil sample."

Altho we seldom fail to get abundant Azotobacter development with sugar, we prefer the starch for several reasons. Gas formation which puffs up the plaques and cracks the smooth surface is greatly reduced; there is practically no trouble with fungous growth; the Azotobacter colonies are better defined and retain their individuality longer with less tendency to spread and coalesce, forming a continuous gelatinous film over the surface of the whole plaque. This is shown in Fig. 9A.

If the sucrose is used in place of starch, we add 1 c.c. of a 100 percent solution of beet sugar to each 50 grams of soil (2 percent). Mannite is employed at the rate of 1.0 percent or 0.5 grams per plaque. While sugar has its objections, as stated, the fact that it can be added as a solution is greatly in its favor when many soils have to be tested, for the time consumed in making numerous weighings of starch is no small item. Again, the Azotobacter colonies seem to develop somewhat more rapidly with sugar than with starch so that readings can be made frequently after 48 hours with the former, while 72 are required usually with the latter. Comparative Azotobacter colony development with starch and sugar is shown in Fig. 9 A, B, C, D.

INOCULATION.—Western arid soils are basic, for the most part, and are inoculated abundantly, naturally, with Azotobacter so that we seldom find it necessary to use a laboratory culture. However if the

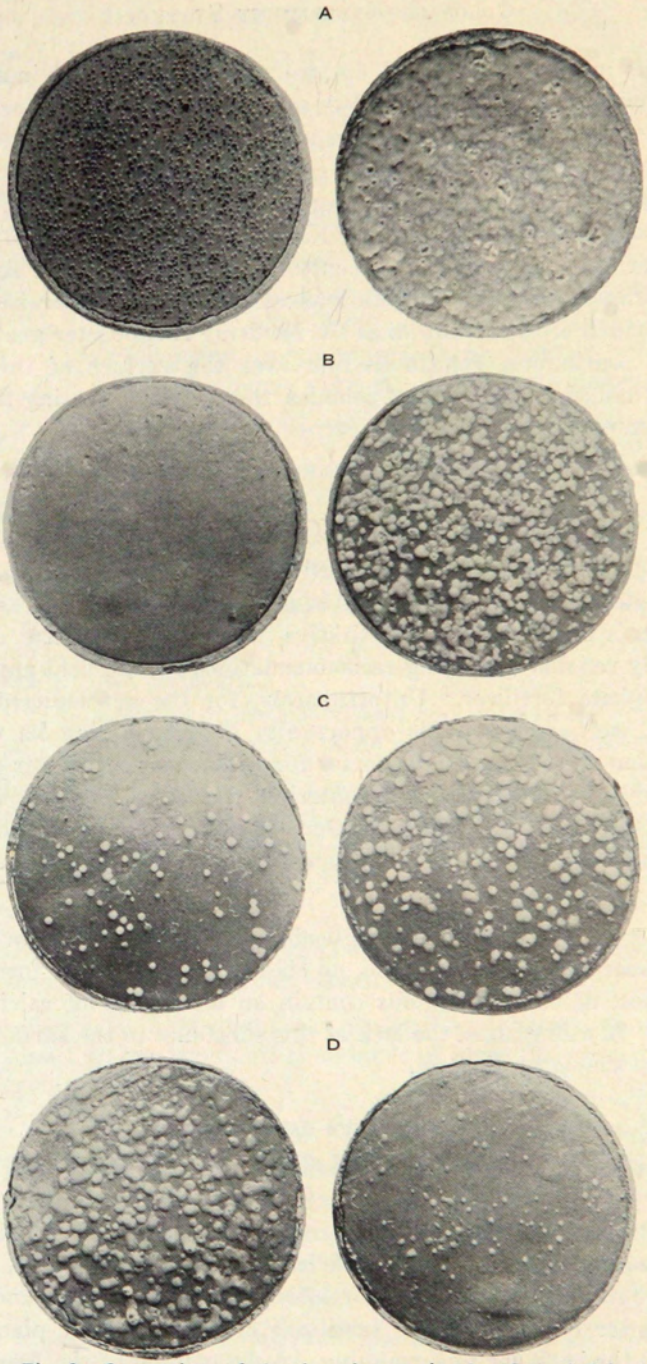


Fig. 9.—Comparison of starch and sugar in phosphate deficient soil. Left column: Starch and phosphate. Right column: Sugar and phosphate.

sample is more acid than pH 6.8, we waste no time and inoculate it at the outset, first having added calcium carbonate to correct the acidity. For this purpose, we use 1 c.c. per plaque of a bacterial suspension prepared by washing the growth from one tube of a 24 to 48 hour mannite agar culture of Azotobacter chroococcum with physiological salt solution (.85 percent NaCl) and diluting this to 100 c.c. with sterile salt solution. The culture is stirred into the soil along with the water and mineral solutions. Care should be exercised not to use too much of the inoculum as the resulting Azotobacter growth may form a continuous, gelatinous film over the surface of the plaque rather than separate, distinct colonies, thereby complicating the interpretation of the test.

Plaques inoculated in this manner are shown in Figs. 2 and 8.

CLASSIFICATION OF DEFICIENCIES

The classification of soils based upon the mineral deficiencies as indicated by the soil plaque test is necessarily a rather flexible one. Altho it is only roughly quantitative, it has proved useful and surprisingly reliable in making recommendations for the field application of phosphate fertilizer. Unfortunately, for the development of the method, we have had little opportunity of determining its value in connection with potash deficiencies for practically all of our soils are still amply supplied with this material. However, thru the courtesy of R. Bach and Co., Stobnitz, Germany, we have obtained several samples of soil low in potash in which this deficiency was clearly indicated by the plaque test.

The method is likewise applicable to the determination of lime deficiencies, but, as with potash, no classification has been formulated, since most of our arable soils contain an abundance of calcium carbonate. In a few cases, the lack of this substance in the surface layers has been shown by the test.

PHOSPHATE DEFICIENCY

Asa Maxson, of the Great Western Sugar Company, conceived the idea of dividing all soils into four groups, based upon their relative mineral deficiencies, corresponding in a general way to the Neubauer Classification, namely: Very deficient, moderately deficient, slightly deficient and not deficient. The relative number and luxuriance of the Azotobacter colonies on the fertilized and unfertilized plaques are taken as the basis for this grouping. A description of the Azotobacter colony development for each of the four classes follows:

Class 1. Very deficient.

Unfertilized Plaque.—Colonies none or few to many extremely small, feeble, pinpoint.

Fertilized Plaque.—Colonies few to numerous, medium to large, distinct and vigorous.

Class 2. Moderately deficient.

Unfertilized Plaque.—Colonies few to as many as fertilized plaque, but very much smaller and weaker in development; none approaching size of colonies on fertilized plaque, pigment often less to none.

Fertilized Plaque.—Colonies few to numerous, distinct and vigorous.

Class 3. Slightly deficient.

Unfertilized Plaque.—Colonies as numerous as fertilized plaque, but smaller and less luxuriant.

Fertilized Plaque.—Colonies few to numerous, distinct and vigorous.

Class 4. Not deficient.

Colonies on both fertilized and unfertilized plaques approximately equal in number and development.

When placing a soil in any one of these classes, it is necessary to take into consideration not only the fact that the fertilized plaque does or does not give better growth than the check, but also the relative number of Azotobacter colonies together with their vigor and luxuriance.

In Fig. 10 are shown typical plaques illustrating the characteristic colony development in the four classes.

In Class I, the untreated check gives no Azotobacter colonies while the fertilized plaque shows an abundance of large, luxuriant, starchy-white colonies.

In Class II, the untreated check exhibits many small, feeble colonies, and the treated plaque shows many large, white, vigorous ones.

In Class III, the colonies on the untreated check are numerous, medium-sized, starchy-white, while those on the fertilized plaque are numerous, starchy-white and somewhat larger and more luxuriant.

In Class IV, both the untreated check and fertilized plaque show numerous, starchy-white to brown colonies, equal in size and luxuriance.

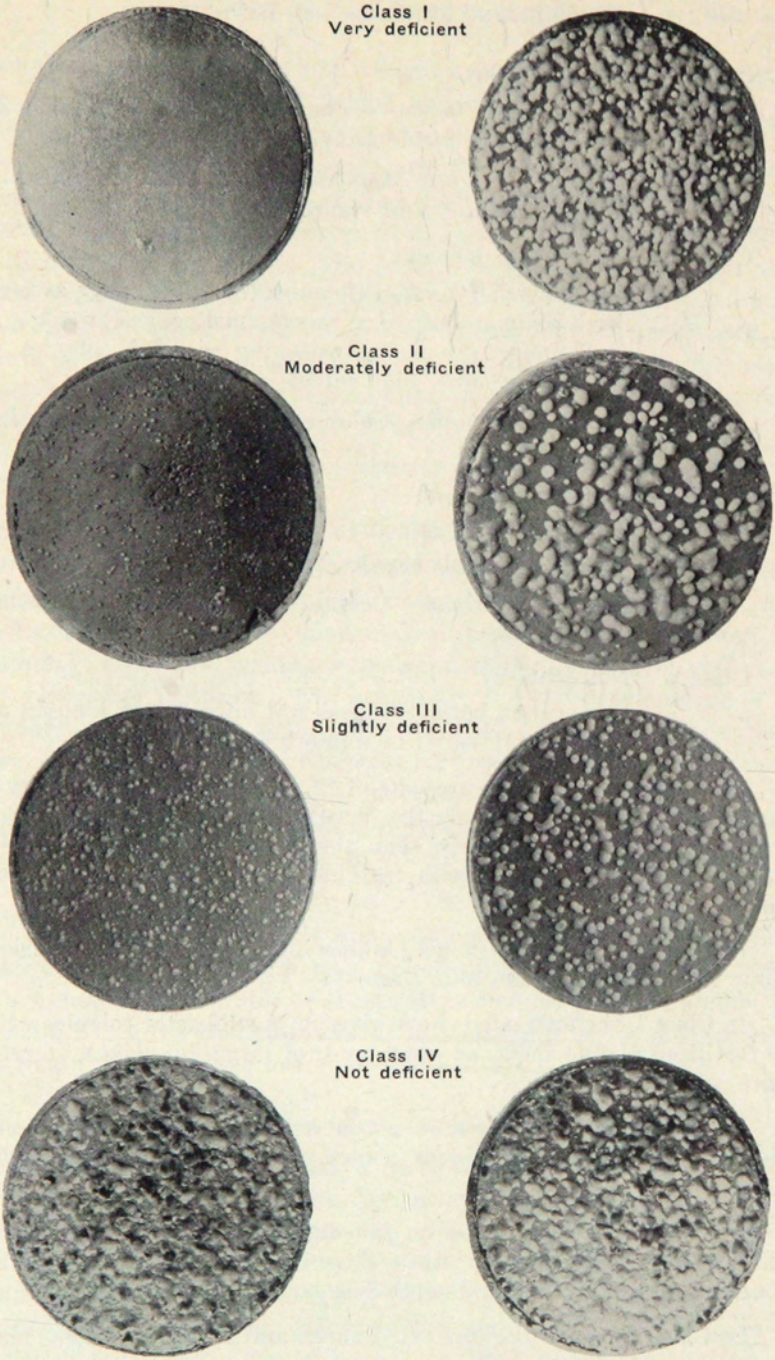
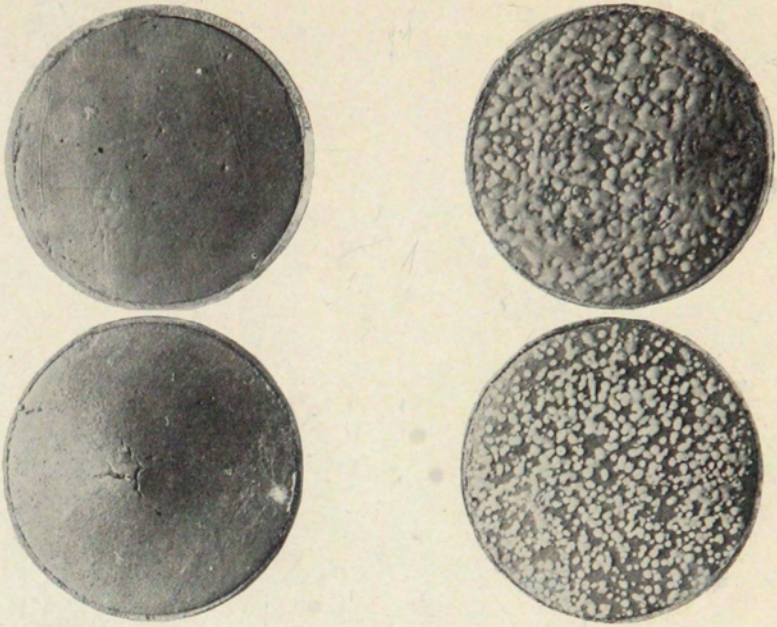


Fig. 10.—Type plaques for phosphate deficiency classification. Left row: Checks, nothing added; right row, phosphate added.

Class I
Very deficient



Class II
Moderately deficient

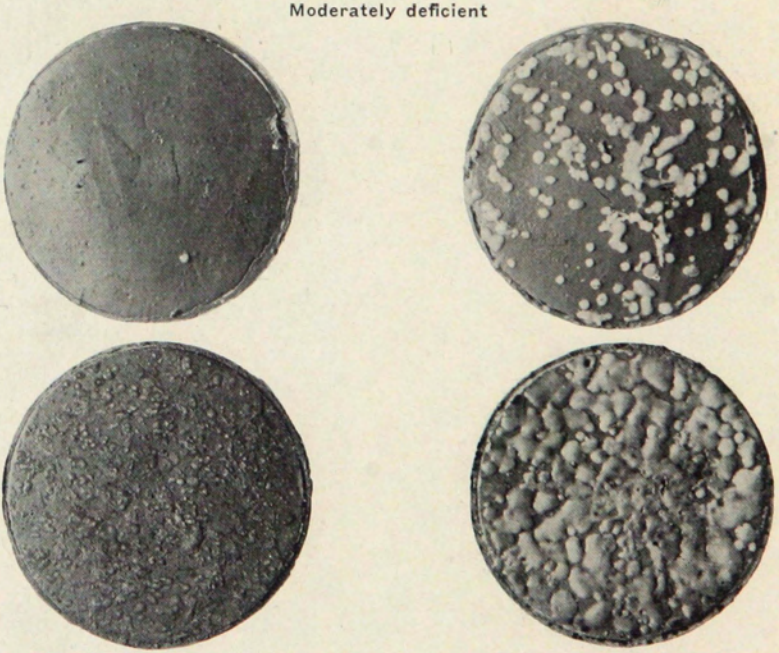
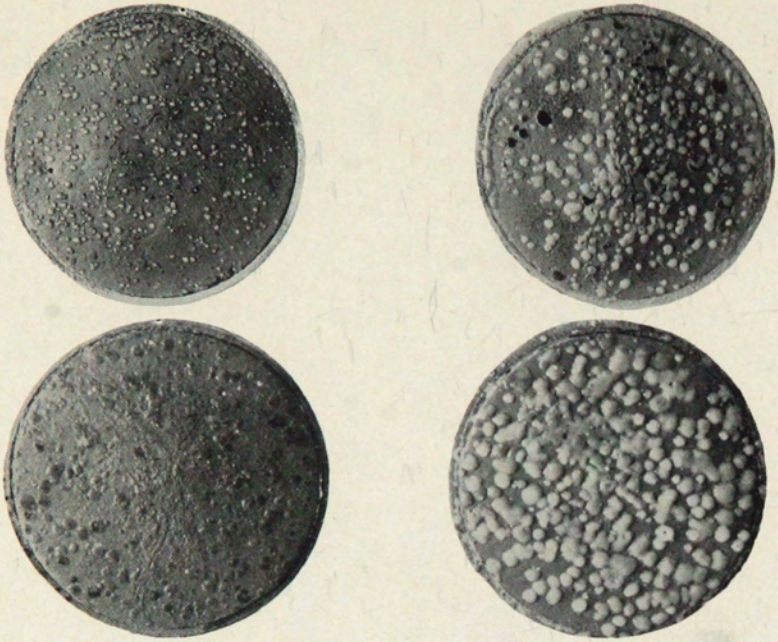


Fig. 11.—Limits of variation for phosphate deficiency classification. Left row: Checks, nothing added; right row, phosphate added.

Class III
Slightly deficient



Class IV
Not deficient

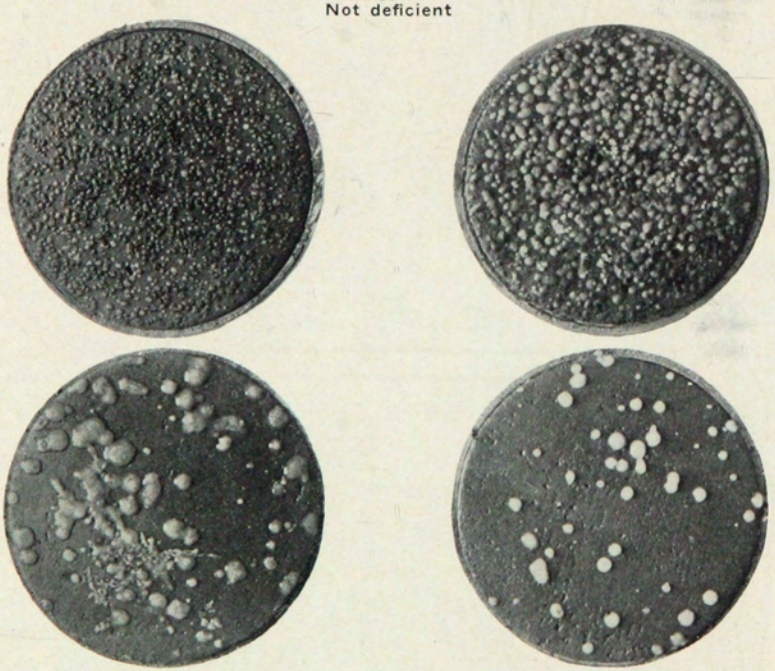


Fig. 12.—Limits of variation for phosphate deficiency classification (continued). Left row: Checks, nothing added; right row, phosphate added.

Obviously all soils will not conform absolutely to these type plaques, and to take care of these variations we recognize certain limits within which the growth may vary for any given class. In other words, there are borderline growths between any two classes which gradually merge into each other. The limits of variation for the four classes as prescribed by our laboratory are illustrated in Figs. 11 and 12.

It will be noted in this connection that in the lower limit for Class I, the check plaque gives no growth whatever, while the fertilized plaque exhibits numerous, large, luxuriant colonies; in the upper limit we observe many extremely small pinpoint colonies on the check and numerous, large, well-defined ones on the fertilized plaque.

In Class II, the check plaque for the lower limit shows many, very small, poorly developed colonies, while on the treated plaque, they are many, large and luxuriant; in the upper limit the colonies are numerous, medium-sized, lacking vigor on the check, and many very large, luxuriant on the fertilized.

In Class III, the check plaque for the lower limit exhibits numerous, medium-sized distinct colonies, while the treated plaque shows the colonies to be as numerous, but larger and more luxuriant; in the upper limit, the colonies on the check plaque are as numerous and almost as large as on the treated, but not so vigorous.

In Class IV, in the lower limit, the colonies on the check plaque are as numerous and vigorous but not so large as on the treated; in the upper limit the colonies on the check are larger and more numerous than on the treated plaque due to the suppression by high phosphate.

While we usually secure a spontaneous development of numerous Azotobacter colonies both on soils which are not deficient and in those in which the limiting factors have been supplied, yet not infrequently we encounter samples which produce only a few colonies even when the deficiencies have been met. We regard this irregularity as due probably to some inherent peculiarity of the particular soil such as the sparse inoculation with Azotobacter or the presence of some inhibiting factors as high nitrates or excessive alkalis.

DISCUSSION OF PLAQUE CHARACTERISTICS

PHOSPHATE DEFICIENCY

In Fig. 3, Soil No. 7, are shown four plaques made from a soil which is very deficient in phosphate. The check plaque to which nothing was added developed no colonies; the second which was fertilized with potash likewise gave no Azotobacter growth, showing that if potash was a limiting factor at all, it was not the only one; on the

third plaque which received the phosphate, numerous, large, luxuriant colonies developed, indicating clearly a benefit from phosphate; on the fourth treated with both phosphate and potash, the colonies were no better than with the phosphate alone, showing that additional potash was not essential and that phosphate was the sole limiting factor. Hence we characterize this soil as deficient only in phosphate. With an application of 200 pounds of treble superphosphate per acre, the yield of sugar beets on the field from which this sample was taken was increased 15.95 percent over the unfertilized check.

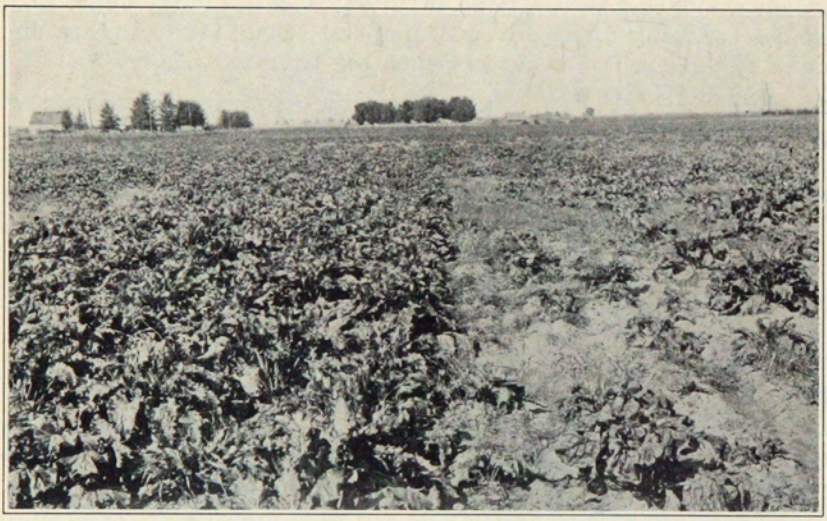


Fig. 13.—Sugar beet field, very deficient in phosphate. Left, 150 pounds treble superphosphate per acre added. Right: Check, nothing added.

Another soil deficient only in phosphate is shown in Fig. 3, Soil No. 44. In this case, the use of phosphate fertilizer increased the sugar-beet tonnage 134.33 percent over the unfertilized area. The field itself is shown in Fig. 13.

No Deficiency

In contrast to the above, Fig. 14 illustrates a field of sugar beets, three-fourths of a mile from the ranch shown in Fig. 13. This soil was not deficient in any of the essential plant foods. It belongs to the same series and to all appearances is the same kind of soil except that it has been handled differently, rotated and manured systematically.

Plaques prepared from this soil are shown in Fig. 4, Soil No. 99. Here it will be observed that all four plaques are practically the same with respect to the number, size and luxuriance of the Azotobacter colonies. We characterize a soil presenting this sort of a picture as

non-deficient. The yield of sugar beets from the unfertilized part of the field averaged 17 tons per acre as compared with 16.68 tons from that fertilized with phosphate.

Fig. 14.—Sugar beet field. Not deficient in phosphate; nothing added.

Another non-deficient soil is shown in Fig. 4, Soil No. 51. Here the average yield for the 20-acre field without any fertilizer was 24 tons of sugar beets per acre.

POTASH AND PHOSPHATE DEFICIENCY

As mentioned elsewhere, there appears to be little or no potash deficiency in any of the soils from Colorado that we have examined thus far.

However, we were able to demonstrate clearly by the soil-plaque method the lack of potash in several samples from Germany known to be low in this substance.

In Fig. 15 is illustrated a set of plaques which we have interpreted as indicating a deficiency in both phosphate and potash. The check gave no growth, showing the absence of one or more limiting factors; the second plaque which received the potash produced no growth, indicating that potash is not the sole deficiency; the third plaque with phosphate grew a few Azotobacter colonies, but they were neither as numerous nor as luxuriant as those on the fourth which received both phosphate and potash. The deficiency was met in part by adding the phosphate alone (Plaque 3), but it was not satisfied entirely until

Sample No. 53

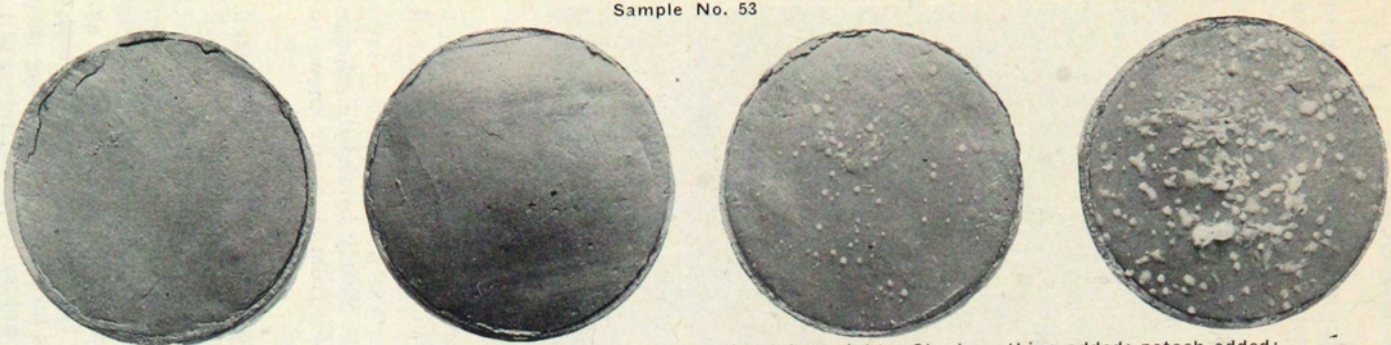


Fig. 15.—Soil deficient in both potash and phosphate. Reading left to right: Check, nothing added; potash added; phosphate added; phosphate and potash added.

Sample No. 105

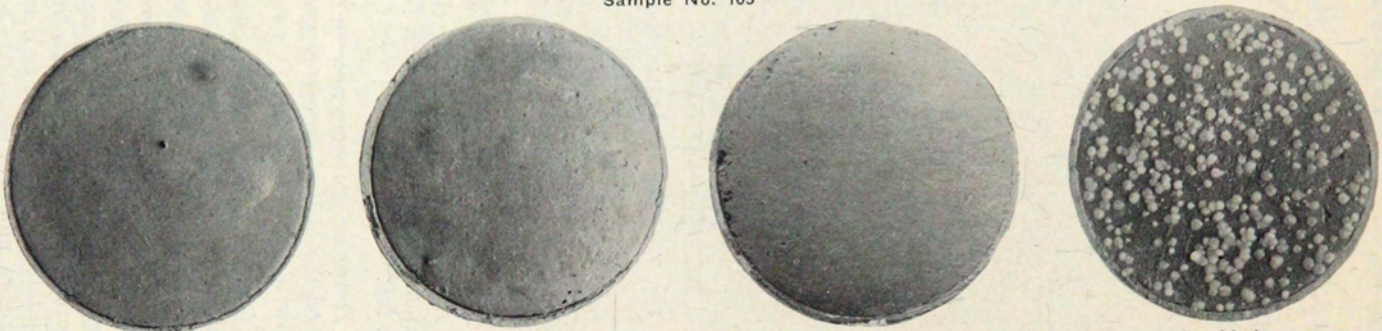


Fig. 16.—Soil deficient both in potash and phosphate. Reading left to right: Check, nothing added; potash added; phosphate added; phosphate and potash added.

both phosphate and potash were used as is evidenced by the improved growth on Plaque 4, hence a deficiency in both phosphate and potash.

The same double deficiency is shown in Fig. 16, but to a greater degree with respect to potash than above. In this case no growth whatever occurred on the check or on either the second or third plaques which received potash and phosphate respectively because both fertilizers were limiting factors. This is indicated by the luxuriant growth on the fourth plaque which was given both potash and phosphate.

SUPPRESSION OF AZOTOBACTER COLONIES BY FERTILIZERS

Both field tests and practical experience have demonstrated the harmful effects of excessive amounts of some commercial fertilizer to growing crops. We have observed the same phenomenon in connection with the development of Azotobacter colonies on soils which are not deficient. The fertilizer added in making the test together with that already present in such soils appears to produce a concentration unfavorable for bacterial growth.

SUPPRESSION BY PHOSPHATE

In Fig. 17 we can observe the suppressing effect of too much phosphate. It will be noted here that the untreated check exhibits numerous, well-developed colonies; the potash plaque is equally good; but, on the third plaque which received the phosphate, there are only a few colonies, and on the fourth where both phosphate and potash were applied there are no Azotobacter colonies at all.

SUPPRESSION BY POTASH

The suppressing action of *potash* is clearly illustrated in Fig. 18. The untreated check produced numerous moderately luxuriant colonies, but the potash plaque gave none whatever; the third which received phosphate shows numerous, vigorous colonies, while the fourth, where both phosphate and potash were used, gave fewer and smaller colonies due presumably to the harmful effect of the potash. It should be stated in passing that a field test with sugar beets and different fertilizer treatments was carried out on this soil. The same depressing action of potash was observed on the beets as was noted with the Azotobacter colonies; the leaves were smaller, light green to yellow in color, the tops were shorter and the yield was 0.64 tons per acre less than on the untreated plot.

Sample No. 92

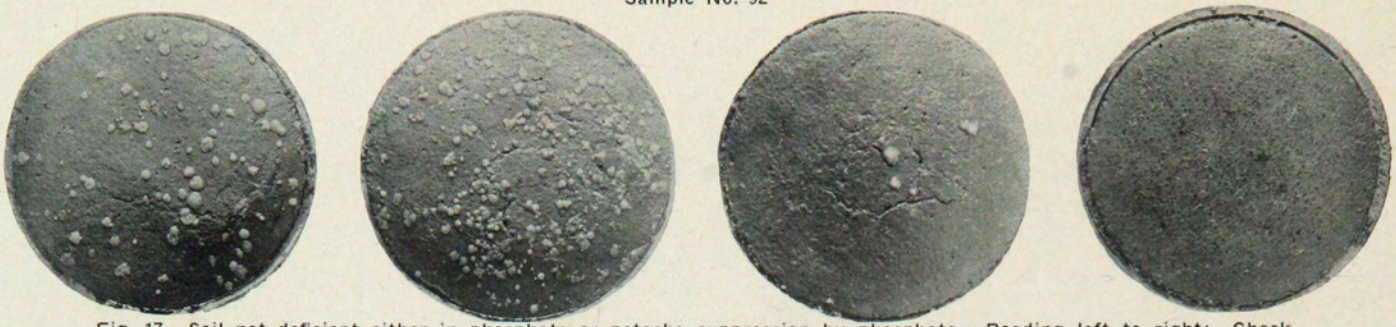


Fig. 17.—Soil not deficient either in phosphate or potash; suppression by phosphate. Reading left to right: Check, nothing added; potash added; phosphate added; phosphate and potash added.

Sample No. 9

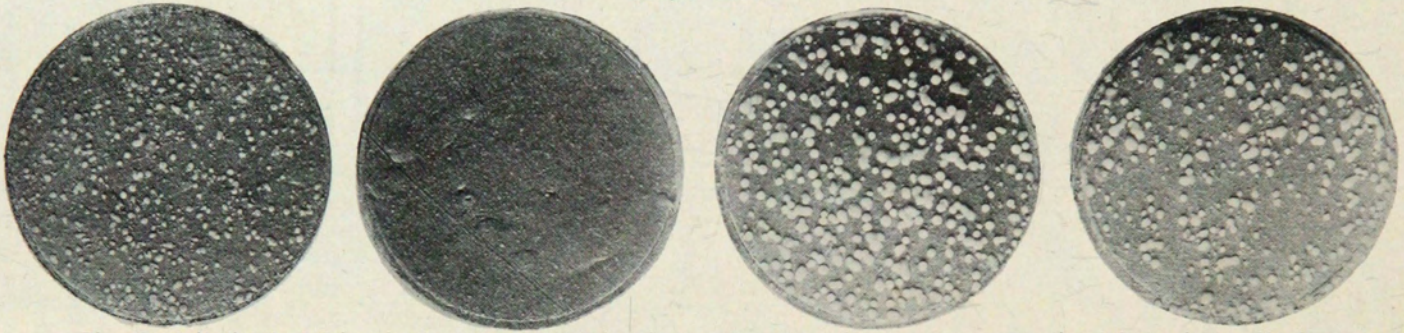


Fig. 18.—Soil slightly deficient in phosphate not deficient in potash; suppression by potash. Reading left to right: Check, nothing added; potash added; phosphate added; phosphate and potash added.

Sample No. 35

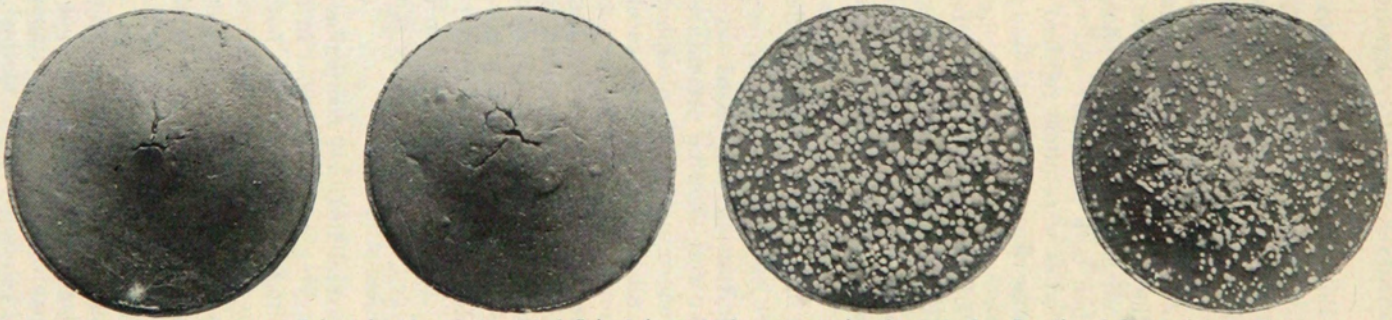


Fig. 19.—Soil very deficient in phosphate not deficient in potash; suppression by potash. Reading left to right: Check, nothing added; potash added; phosphate added; phosphate and potash added.

SUPPRESSION BY POTASH WITH DEFICIENCY IN PHOSPHATE

In Fig. 19 we have an example of a soil which is very deficient in phosphate, but which contains an abundance of potash, so much of the latter, in fact, that harm results when more is added. The untreated check gave no growth indicating the need for something; the potash plaque produced no colonies either, showing that potash was not the only limiting factor; the phosphate plaque gave numerous, luxuriant Azotobactor colonies, indicating clearly that phosphate was the principal deficiency; but, when both phosphate and potash were added, as in plaque four, the colonies were much reduced both in number and size due to the depressing effect of the potash.

PRACTICAL APPLICATION OF THE PLAQUE METHOD

Ziemiecka (7), (8) is among the first investigators to make practical use of the soil plaque in determining mineral soil deficiencies. She employed it successfully in her studies of the phosphate and lime needs of Polish soils.

Niklas (4) used it in the determination of the lime and phosphate requirements of certain German soils.

More recently, Guittoneau (2) tested several soils from plum orchards in France that were showing premature mortality. He used the plaque for the determination of phosphate, potash and lime deficiencies and found all but his fertilized check orchard to be very low in available phosphoric acid. No need for potassium was indicated which could be explained by the fact that these soil types are not particularly low in this element. The results obtained agreed with the chemical tests made previously which had shown low available phosphoric acid. Regarding the value of the method, Guittoneau states, "The Azotobacter method has confirmed the cultural indications directly gathered on the grounds. It confirmed also the information that we had obtained already, but very much more laboriously elsewhere, by our chemical studies."

In this country, several fertilizer companies and agricultural experiment stations are trying out the method in an experimental way on a small scale according to our procedure, but probably the widest practical application to field testing has been made by the beet-sugar companies operating in Colorado, Wyoming, Nebraska, Montana, Utah and Idaho. During the past 2 years, literally thousands of soil samples have been tested for phosphate deficiency in their laboratories, using the technique developed in our laboratory. Because of the large number of samples examined and the opportunity afforded to follow up the results of fertilizer application based upon these tests, the data collected by these companies are of greatest importance in arriving at the practical value and reliability of the method.

Mr. Maxson, speaking for the Great Western Sugar Company, informs us that in 1930, he secured a 92 percent correlation between the plaque test and the field results as shown by the sugar-beet yields where phosphate was applied as indicated by the test. He states further that:

"The field trials of this company for checking plaque tests against percentage of fields giving gains with phosphate, show 93 percent of Class 1, 88.6 percent of Class 2, 86.2 percent of Class 3 and 75 percent of Class 4 giving gains. The size of these gains was on the average, 2.29 tons per acre for Class 1, 1.51 tons for Class 2, .91 tons for Class 3 and .77 tons for Class 4."

The use of phosphate is not recommended on a field scale for soils which fall into Class 3 since profitable increases are not always obtained where only a slight deficiency exists. In these cases, it is usually suggested to the grower that he try the fertilizer on a small area before making a general application.

For moderately deficient soils, 100 to 125 pounds of treble superphosphate per acre are recommended; for very deficient, an application of 125 to 150 pounds are advised.

Regarding the results obtained by the Utah-Idaho Sugar Company with the soil plaque in determining phosphate deficiencies, Douglas Scalley, Utah District Manager for this company, has written us as follows:

"For the year 1930 we made a very close tabulation of our results by the soil-plaque method of phosphate requirements on the beet soils of Utah and Idaho, and our final tabulation shows a little over 96 percent of the farms that were treated with phosphate, responded to the treatment. This response varied from 1 ton to 10 tons per acre. The districts which showed the greatest increase in tonnage thru the use of phosphate were where we were troubled with the beet leaf hopper and many fields treated with phosphate in these districts harvested over an 8 to 12-ton crop while many fields not treated were not harvested. We have noted the same response to the alfalfa and potato crops in the sections where phosphate has been used."

Our first studies with the soil plaque as a means of determining mineral soil deficiencies were begun in the summer of 1927. The routine examination of field samples by this procedure was inaugurated in the spring of 1929.

Since any method of determining soil requirements, to have a practical value, must give results which can be verified by fertilizer field tests with growing crops, arrangements were made with Dr. D. W. Robertson of the Agronomy Section of the Experiment Station to carry on cooperative fertilizer field experiments in 1929, 1930 and 1931 on land which we had tested for deficiencies in phosphate, potash and lime. Twelve farms, representing the Fort Collins and Campion Series, were selected for this work. Eight of these needed phosphate,

while none lacked either potash or lime according to our tests. Sugar beets were planted for the experimental crop. Each set of experiments included treatments with treble superphosphate, potassium sulphate and a combination of these two together with the untreated checks. The detailed results of this work have been reserved for a subsequent publication, but it will suffice for our present purpose to say that phosphate was the only material that proved beneficial. Where this was used on the eight deficient soils, increases ranging from 13.43 to 134.33 percent in the sugar-beet yield were obtained; where there was no deficiency, there was no response.

During the past 2 years we have examined more than 600 individual field samples and in a number of cases we have been able to follow our recommendations thru to the harvest. In practically all of these, where the deficiency indicated by our test has been supplied, there has been either an increase in the yield or an improvement in the quality of the product.

SUMMARY

The response which many Colorado soils give to barnyard manure and commercial fertilizers indicates that the supply of available plant food originally present in the virgin soil is becoming depleted, and that if we are to maintain our soils in a high state of productivity, steps must be taken soon both to conserve and restore the lost fertility by systematic rotations, green and barnyard manures and commercial fertilizers. It is important to determine by a suitable soil test what these deficiencies are and where they exist so that the proper fertilizers can be applied where they will do the most good and not be wasted where they are not yet needed.

The soil plaque which utilizes the spontaneous development of Azotobacter colonies as a plant-food indicator has proved to be a satisfactory means of determining deficiencies in phosphate, lime and probably potash. The method is briefly this: Four plaques are prepared from each soil under examination, 50 grams being used for each plaque. Energy material in the form of either cornstarch or sucrose is added to all of the plaques. The first of these is reserved without further treatment for a check, the second receives K_2SO_4, the third Na_2HPO_4 the fourth K_2HPO_4 and a fifth plaque with $CaCO_3$ may be made to test for lime deficiency. Sufficient distilled water is added to each to make a soft, plastic mass which is transferred to a small culture dish and molded into a smooth-surfaced plaque. The four plaques, which constitute the set, are placed in a moist chamber and incubated for 72 hours at 30 degrees C. At the end of this period, Azotobacter colonies, visible to the naked eye, will have appeared on the plaques where the deficiency has been satisfied or where none existed, while no growth will be present where the proper limiting

factor has not been supplied. The relative colony development on the untreated check and the fertilized plaques indicates the degree of the deficiency. Four classes of phosphate deficiency corresponding to the Neubauer Classification have been established.

Fertilizer field experiments, based upon soil-plaque tests, have shown the accuracy and value of the method.

CONCLUSIONS

The bacteriological soil plaque offers a rapid and dependable method for determining phosphate and lime deficiencies in soil, and may prove equally useful for potash.

The test is not only qualitative, showing the mineral elements needed, but also is sufficiently quantitative to indicate, with sufficient accuracy for all practical purposes, the amount of fertilizer to apply in the field.

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