The Winogradsky test for determining plant food deficiency in Montana soils
by Charles Robert Johnson

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Montana State University
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Abstract:
1. The most Important advantages of this test are: (a) the short time required for its completion, (b) greater control of external factors which limit the value of results obtained from field experiments (c) relative Inexpence of the method, (d) a great number of samples can be tested before the planting season, (e) close correlation of the teat remits to field results.

2. Seven-hundred and fifty samples sere tested over a two year period and 65% of them showed a phosphorous deficiency ranging from moderate to very deficient, 22% showed a slight deficiency, 10% showed no deficiency, end 3% wore indeterminate.

3. The correlation of the Rinogradsky test and the field results in the esse of sugar bests was 95%. Soil tested from a phosphate plot after a crop of sugar beats had been renewed showed a decided phosphate deficiency, indicating that the sugar beets removed a good deal of the phosphorous applied.

4. The correlation of the Winogradsky teat and field results In the ease of alfalfa was 80% over the two year period. The content of phosphorous In the plant increased In proportion to the increase In yield of alfalfa.

5. The correlation of the laboratory and field results fbr Irrigated Wheat fbr the 1931 season was 73%. In some cases the grain on the phosphate plot matured from a week to ten days earlier than the grain on the check plot.

6. No correlation between laboratory and field results was observed In the ease of beans. It appears that a good yield of beans can be obtained from a soil low In phosphorous.

7. The percentage of Montana soils deficient In potash is very low. Only one soil showed a deficiency of potash alone. Fifteen per cent of the soils Whowed a deficiency Cf potash In combination with phosphorous.

8. Many western Montana soils appear to be lacking in Azotobacter and are also acid In reaction.

9. Inoculation of a soil containing no Azotobacter by a soil known to contain many Azotobaeter proved In general more satisfactory than the Inoculation of the soil by a pure eul tore of Aaotobacter.

10. Acid soils were brought to the neutral point by the addition of CaCO5. Neutralization of the soil plus inoculation brought positive results from previously Indeterminate soils. In some cases where the soil was neutral inoculation alone gave positive results.

11* Gypsum (CaSO4) particularly In ease red soils from Sanders county produced good Azotobacter growth when used In combination with phosphorous, where phosphorous alone gave no results* 18* One hundred samples were tested by the Truog phosphorous method end no definite correlation was found between the Truog and Wlnogradahy methods regarding phosphorous availability* The exact

reason for this leek of correlation is undetermined* 13* Sugar used In place of corn starch as a source of energy fbr the bacteria, produced a film over the surface of tine soli plaque, making an estimation of the number and type of the bacterial growth Impossible. Sugar failed to produce growth when used In a peat a oil, Alch also did not respond to starch*

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by

CHARLES R. JOHNSON

Walsh Research Fellow in Agriculture, 1931-32

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INTRODUCTION

The problem of determining the plant food deficiencies of soil is gradually increasing in importance, due to some extent to the fact that our extensive frontiers with their countless acres of virgin soil have practically all disappeared. The problem of soil fertility can no longer be solved by moving to a new section where the soil still retains its natural fertility.

A great number of the once productive farms of New England, and the Southern and Middle Western states are today practically worthless, because they were cultivated until they reached a depleted condition. The cost of restoring them being almost prohibitive.

Montana soils are much nearer the virgin state than those mentioned above, and have until recently produced exceptionally well. Although it has been noticed for a number of years that an application of barnyard manure on Montana soils increased production. It appears now that many Montana farms have arrived at the point where the reserve of some of the mineral plant food in an available form, has been consumed, and that the crop demands are exceeding the supply, the result being a noticeable reduction in yields in recent years.

The problem of determining these deficiencies of the soil is probably not one of soil type or locality, but of the individual field, depending upon the crop history and general method of farming.

The Winogradsky bacteriological test for mineral deficiencies

of soil (especially regarding phosphate deficiency) has come into prominence. The purpose of this work was to determine the applicability of the test to Montana soils, and to overcome previous difficulties encountered in testing Montana soils by this method.

The work was done in the bacteriology laboratory of the Agricultural Experiment Station of the Montana State College in 1931-32 under the supervision of Professor H. E. Morris and Dr. D. B. Swingle, in cooperation with the Chemistry Department of the Agricultural Experiment Station. It was a continuation of studies begun as a bachelor's thesis and was made possible by the provisions of the Senator Walsh Research Fellowship.

METHODS OF TESTING SOIL FOR MINERAL DEFICIENCIES

Neubauer Test

Rye plants are grown in the laboratory for 14 days upon the soil under examination, and from a chemical analysis of the plants the shortage of phosphate and potash is determined (12). Because of the time and expense involved it would not be feasible to test any great number of samples before the planting season. However, the results obtained from this method appear to be quite dependable.

Chemical Analysis

The chemist in his soil analysis by colorimetric methods employs weak acids as solvents in an effort to imitate the action of plant roots in dissolving the mineral particles. This gives only an approximate idea of the amount of material which is in a form that plants can

utilize. The mineral food which a growing plant can utilize and that which is dissolved by chemical methods may be quite different. A chemical analysis, therefore, is valuable only where a marked deficiency or excess exists, and to show the potential amount of certain minerals in the soil.

Experimental Plots

Different fertilizers at varying rates are applied to experimental plots which are then planted to growing crops. At the end of the season, yields are taken and conclusions drawn as to the relative benefits of the treatments. This method is limited in its general application, and possesses little value for the individual farmer, unless he has an experimental plot for each field. This point is understood when consideration is given to the fact that every field has its own deficiencies, depending upon crop history and farming practices with regard to that field.

Colorimetric Methods for Determining Phosphorous

In 1920, Deniges (5) published a method for the colorimetric determination of phosphorous present as phosphate, by means of a blue color produced when a reducing agent is added to an acid solution of ammonium molybdate containing inorganic phosphates. The above reaction is the basis for colorimetric methods for determining phosphorous.

Chapman (3) states, regarding the colorimetric methods of determining phosphorous in soils, that there is a necessity for further

work regarding the possible influence of such concentrations of salts as may be found in the soils and irrigation waters of semi-arid regions on the accuracy of tests of this type. He further states that silicon dioxide and the ferrous ion, as well as nitrates and sulphates under some conditions influence the results of this test.

Hibbard (7) states the following regarding the Truog colorimetric method: (19) "of all equilibrium methods Truog's is perhaps the simplest, cheapest, quickest and generally the most useful. It has the defects of other equilibrium methods, and also the large portion of solvent to soil used in this method has a leveling effect, tending to make poor soils seem better, and rich soils seem poorer. He also states that the K_2SO_4 used in the Truog solvent changes the solubility of PO_4 in some soils more than in others, again tending to misrepresentation."

Winogradsky Soil Test

Historical

Greaves (6) gives the following historical account: "The first one to hint at the fact that microorganisms in the soil might take part in the process of nitrogen-fixing was Boussingault in the middle of the nineteenth century. Thirty years later Hellriegel and Wilforth made the discovery of nitrogen-fixing by symbiotic organisms. In 1883 Berthelot proved conclusively the presence in the soil of chlorophyll-free bacteria capable of fixing atmospheric nitrogen. This

discovery interested Winogradsky, who worked with fifteen separate species of soil bacteria and discovered that the power of fixing nitrogen was not general among the soil microorganisms, but confined to a few special forms. Following this, Baron did much work with a spore-bearing bacillus. In 1901, Beijerinck furthered the history immensely by his discovery of a new group of large aerobic bacilli which he called Azotobacter. He, working with von Delden, believed that the nitrogen-fixing power of Azotobacter depended on other soil organisms. Later Gerlach and Vogel isolated one of Beijerinck's Azotobacter in pure culture and showed that it was capable of active nitrogen-fixation.

Beijerinck and von Delden had described A. chroococcum and A. beijerinckii, and A. woodstownii. Later Lohnis & Westermann described A. vitreum. All workers have found A. chroococcum to be the most widely distributed in the soils so far studied."

A test which gives accurate information as to available plant food and requires a short enough time to be of practical value was worked out by Winogradsky and Ziemiecka of the Pasteur Institute, of Paris, Franco. These workers discovered that the plant foods such as phosphate, potash, and lime, which are essential to plant growth, are also essential to the growth of the Azotobacter, nitrogen-fixing bacteria, and that these bacteria will not grow where the soil is deficient in these foods. Therefore, different fertilizers, singly or in combination, are added to the soil samples and the results