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TRIALS WITH A BACTERIOLOGICAL METHOD FOR MEASURING AVAILABLE PHOSPHORUS IN SOILS

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The basis of most laboratory tests for determining the availability of plant food constituents in soils is the assumption that the materials dissolving in some particular solvent are available for plant use. Crop growth, however, has not always been correlated with the solubility of the constituents and various solvents have been employed in the attempt to obtain a definite correlation. One of the first methods proposed for determining the availability of phosphorus in soils consisted in the determination of the phosphorus extracted from the soil by a 1 per cent solution of citric acid. It was claimed that the root sap of most plants and a 1 per cent solution of citric acid were about the same in acidity, hence, this solution should extract about the same amount of phosphorus from the soil as the plant would be able to obtain. A 0.002 N sulfuric acid solution has also been proposed as the extracting solution. Both methods correlate fairly well with crop response to phosphate fertilizers on some soils but neither method is satisfactory for all soils.

It has been claimed that the Azotobacter make more growth in a soil well supplied with available phosphorus than in a soil deficient in phosphorus. A method for determining the phosphorus needs of the soil, sometimes referred to as the "mud pie" method, is based upon this assumption. Truffaut and Bezsonoff[1] found that the amount of nitrogen fixed by species of Azotobacter, Bacillus and Clostridium in a nitrogen — poor solution was related to the amount of phosphorus in the medium. It was found that a gain of 2 mgm. of nitrogen by a mixed culture of these bacteria corresponded to the assimilation of 1 mgm. of phosphorus. It was suggested that the amount of nitrogen fixed by a mixed culture of these organisms growing in a solution culture with soil as the sole source of phosphorus would serve as a measure of the availability of phosphorus in that soil.

The purpose of the work reported in this paper was to determine

[1] Truffaut, G. and Bezssonoff, N. 1928. Methode bacteriologique permittant la determination du taux d'assimilabilite des phosphates. La. Sci. du sol. 7:1-38.

the availability of phosphorus in several soils by the bacteriological method suggested by Truffaut and Bezsonoff for comparison with crop response to the fertilizers and the results obtained by a chemical method using 0.002 N. sulfuric acid as the extracting solution.

A slightly acid Carrington loam was used. The soil was brought into the greenhouse and sieved through a one-fourth inch sieve. Ammonium sulfate and potassium chloride were each applied at the rate of 200 pounds per acre to all soils. Twenty 4-gallon pots were filled with this soil. Two pots of soil were left untreated and served as a check. The soil in duplicate pots was treated with 300 pounds per acre of superphosphate. The other 16 pots of soil were treated in duplicate with different phosphate fertilizers on the basis of an equivalent amount of phosphorus to that supplied in the superphosphate. The fertilizers were thoroughly mixed with the soil and the moisture content of the soil adjusted to approximately 50 per cent of the saturation capacity. This moisture content was maintained throughout the experiment by additions of distilled water.

Spring wheat was planted March 20. The wheat made an unsatisfactory growth and showed the symptoms of nitrogen starvation. Four grams of sodium nitrate in 100 cc. of water were added to each pot April 2. The wheat was harvested May 26. The plants were dried at 70° C. and weighed. The results are given in Table I and represent the averages of duplicate pots.

The soils were sampled after the wheat was harvested and the amount of available phosphorus determined by the 0.002 N. sulfuric acid method and by the method of Truffaut and Bezsonoff modified as noted. Five-tenths gram of soil which served as a source of phosphorus for the bacteria was added to 50 cc. of a culture medium containing 0.08 per cent KCl, 0.02 per cent NaCl, 0.02 per cent $MgSO_4$, 0.0002 per cent nitrogen as egg albumin, 0.4 per cent $CaCO_3$ and 1 per cent glucose, sterilized at 15 pounds pressure for 15 minutes, cooled and inoculated with *Azotobacter vinelandii, Bacterium radiobacter* and *Clostridium pasteurianum.* Truffaut and Bezsonoff recommended inoculation with *Azotobacter agile, Bacillus truffauti* and *Clostridium pasteurianum.* After incubation at 35° C. for 21 days, total nitrogen was determined by the Kjeldahl method and the corresponding amounts of P_2O_5 calculated. The results obtained by the two methods are given in Table II.

The data in table I indicate no beneficial effect of the fertilizers on the yield of wheat. The fertilized soils yielded less wheat than

Table I — Effect of Various Phosphate Fertilizers on the Yield of Spring Wheat

No.	Fertilizer	Yield of grain (gms.)
A-0	Check	22.0
A-4	Superphosphate (high in Fe and Al)	16.3
A-10	Ammoniated superphosphate (2.48% ammonia)	21.8
A-12	Ammoniated superphosphate (6.83% ammonia)	18.0
A-13	Ammoniated superphosphate (8.66% ammonia)	20.0
B-4	Tricalcium phosphate (15.94% citrate-sol.)	17.6
B-5	Tricalcium phosphate (13.21% citrate-sol.)	14.5
B-6	4 parts tricalcium phosphate (21.24% citrate-sol.) 1 part monoammonium phosphate	17.5
B-13	Natural Fe and Al phosphate	12.4
B-14	Tennessee brown rock phosphate	10.9

Table II — Milligrams P_2O_5 per 100 grams Dry Soil

No.	Fertilizer Treatment	0.002 N. H_2SO_4	Assimilated by bacteria
A-0	Check	2.98	54.0
A-4	Superphosphate (high in Fe and Al)	2.98	48.0
A-10	Ammoniated superphosphate (2.48% ammonia)	2.98	46.0
A-12	Ammoniated superphosphate (6.83% ammonia)	3.39	44.0
A-13	Ammoniated superphosphate (8.66% ammonia)	3.66	44.0
B-4	Tricalcium phosphate (15.94% citrate-sol.)	2.98	40.0
B-5	Tricalcium phosphate (13.21% citrate-sol.)	2.98	36.0
B-6	4 parts tricalcium phosphate (21.24% citrate-sol.) 1 part monoammonium phosphate	3.48	50.0
B-13	Natural Fe and Al phosphate	3.62	18.0
B-14	Tennessee brown rock phosphate	3.98	38.0

the unfertilized check. This was undoubtedly due to other conditions than the lack of available phosphorus.

The results in Table II show no correlation between the amount of available phosphorus in the different soils by the two methods. The check soil contained the highest amount of available phosphorus by the bacteriological method, whereas by the 0.002 N. sulfuric acid method, this soil was among the lowest in soluble phosphorus.

The presence of *Bacterium radiobacter* in the culture, the substitution of *Azotobacter vinelandii* for *Azotobacter agile* as well as the absence of *Bacillus truffauti*, may be responsible for the lack of agreement between the two methods. However, *Bacterium radiobacter* occurs in the soil closely associated with species of Azotobacter. The ability to fix nitrogen has been claimed for the radiobacter but this has been questioned. However, it has been shown that Azotobacter may fix more nitrogen in a mixed culture of *Bacterium radiobacter* than in pure culture.

These results are too meager to warrant the drawing of conclusions, and further work is necessary but the results of other experiments obtained in the authors' laboratory would seem to indicate that the fixation of nitrogen by the Azotobacter is not a function of the phosphorus assimilated. This work is being continued for further comparison with results on crop response to fertilizers in field and greenhouse studies.

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