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A COMPARISON OF THE SPECTROPHOTOMETRIC MICRO-DETERMINATION
OF INORGANIC PHOSPHORUS BY THE DENIGES METHOD AND
MOLYBDENUM BLUE METHOD WITH APPLICATION
TO SOIL EXTRACTS

by

HIRAN TEMPLE MCGEEHEE

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INTRODUCTION

An unsatisfactory feature of the blue colorimetric method for determination of available inorganic phosphorus in soil is the yellow color, which occurs in the displaced solution and water extract of many soils. Soils that give this yellow color are "black alkali" soils, of some regions, and in soils where the exchange complex is saturated with Mg^{++} , Na^+ , K^+ , or NH^+ . This work was begun with the hope of finding some method to eliminate the effects of this yellow color on the determination.

There are also some questions concerning the best method to determine small amounts of phosphorus in soil extracts. A study of the color intensity of two methods has been made. The methods are the ammonium molybdate method of Deniges and the molybdenum blue method of Zinzadze. The effects of acids, bases, and ferric iron were determined upon the molybdenum blue method.

Different instruments have been used to determine the intensity of the blue color. The most commonly used instrument is the colorimeter. In this instrument, a standard solution of known concentration of phosphorus must be prepared. This must be prepared fresh every two or three days or protected from bacterial action with a layer of some substance, (7) toluene being the most often used.

Trouble is encountered here, because the phosphorus in the standard emulsifies the toluene, causing interference in the matching of the color. The spectrophotometer may be used in determining the intensity of the blue color also.

The use of the spectrophotometer is based upon light absorption. When light passes through any homogeneous transparent medium, it emerges diminished in amount. Part of the light may be scattered at the surface, part scattered in the interior, and part regularly reflected at the surface. The rest of the light which is lost is said to be absorbed. This is the portion of the light that is of interest in this problem. Allowance must be made for the other lost light in taking measurements. This is taken care of by such arrangement as passing the comparison beam through an exactly similar optical path including the colorless solvent always the same depth as the sample. In this work the blue color was developed in the soil extract and another sample was made to the same acid concentration. The latter sample was used to counteract the effects of the yellow color of the soil extract, as well as the light lost in other ways. This made it possible to measure only the light that was absorbed by the molybdate compound.

The two principal laws of light absorption are those of Lambert and Beer. Beer's law states that if an absorbing substance is dissolved in a non-absorbing liquid,

its absorption of a beam of homogeneous light depends on the number of molecules of the absorbing substance which the beam of light passes through, i.e., on the concentration of the solution.

Lambert's law states that the proportion of light absorbed by a substance is independent of the intensity of the incident light. This law according to Twyman (22) is rigidly true. Lambert's law may be expressed by the following formula:

$$k = \frac{1}{d} \ln \frac{I_0}{I}$$

where k is a constant and is the absorption coefficient, which depends on the nature of the material and the wavelength of light, d is the thickness, I is intensity of light after passing through a uniformly absorbing medium, I_0 is incident light or intensity of light that has passed through the undeveloped solution.

This equation is often written with common logarithms, and in place of constant k , a constant ϵ is used. The equation becomes:

$$\epsilon = \frac{1}{d} \log \frac{I_0}{I}$$

where ϵ is called the extinction coefficient; it may be defined as the reciprocal of that thickness which reduces

the intensity of the transmitted light to one-tenth of its original value.

There is another term which is often used, namely, transmittancy. Transmittancy is defined as the ratio of the radiant power incident on the second inner surface of the cell to that passing the first inner surface. Then absorption = $1 - T$. Where $T = \frac{\text{Transmittance of solution}}{\text{Transmittance of solvent}}$ or

$$\epsilon = -\frac{1}{dc} \log_{10} T$$

where ϵ and d have same meaning as mentioned above and c represents concentration. Concentration is reported in this thesis as parts of P_2O_5 per million. Transmittancy of a cell containing the solution is always compared with that of a cell containing the solvent to the same depth. Hence, transmittancy is the fraction of light transmitted by a solution of given thickness and concentration.

The problem then to make a quantitative determination of the blue color with the spectrophotometer is to find the wave length of maximum absorption and to determine extinction coefficient at this wave-length.

Teorell (3) in his investigation of the Fisk-Subbarow method has reported that the ratio between the phosphorus content and extinction coefficient for this method to be .194 at a wave-length of 720 $m\mu$.

Because of the common use in the literature of reporting phosphorus as parts per million of P_2O_5 the extinction coefficient is reported using this notation with the depth of solution at one centimeter.

REVIEW OF RELATED LITERATURE

The fact that a blue color is produced when the molybdenum in the complex phospho-molybdate solution is partly reduced is far from a recent discovery. Osmond, in France, (1) published (1887) some work on this subject. Osmond precipitated the phosphorus in the ordinary way and filtered it on an asbestos filter. The precipitate was dissolved in a hydrochloric acid solution of stannous chloride. The volume was made up of 100 cubic centimeters and compared to a standard made in like manner or a disc of colored paper of known intensity.

Taylor and Miller (15), in this country, also noticed this phenomenon, i.e., a blue color produced when a reducing reagent was used. They suggested the possibility of using this reaction as a basis for the colorimetric determination of phosphorus. Subsequently, Wu (9) made a detailed study of the chemistry of the reaction, but did not develop a quantitative method. Deniges (11), Bell and Doisy (12), Briggs (13), and Fiske and Subbarow (14) developed methods for quantitative determination of phosphorus.

A comprehensive review of the literature on the above methods shows that the blue color increases in intensity for a short time then fades. This is thought to be due to oxygen. Truog and Meyers (7) tried to prevent this by adding substance that would prevent the oxygen from coming in contact with the solution. Gelatine was the only thing that prevented the color from fading, but this interfered with the matching of the color. Chapman (8) has shown that when .6 mg. of $\text{Sn Cl}_2 \cdot \text{H}_2\text{O}$ in 100 cubic centimeters of solution containing 3.2 parts per million of PO_4 gives a uniform color for one hour. Where more of the stannous chloride would give a greater intensity of color, but would fade after standing 15 minutes.

Zinzadze has worked out a blue colorimetric method for the determination of phosphates and arsenates in solution. The reagent for this method is made by dissolving Mo O_3 and molybdenum metal in sulfuric acid. The directions for making up the reagent will be given later.

According to Zinzadze this new method is superior to other methods in two ways: (1) The reagent is preserved for a very long time without any change. (2) The blue coloration given by phosphates and arsenates does not change in a long time. If the solutions are kept in the dark, the color stays stable for seven to ten days. In solutions remaining

in the light, the color begins to fade in two to three days. This method also has an objection; the color develops very slowly. Maximum color is developed in two to three days at room temperature or eight minutes at boiling temperature.

Different instruments have been used to match these colors. The most common instrument used is the colorimeter and matching against a standard of known phosphate concentration. Chapman (6) used a rotating color disc, which was calibrated against known concentrations of phosphorus. Teorell investigated the Fiske-Subbarow method with the spectrophotometer with relation to blood serum. He worked out the extinction coefficient for this method. He shows that this method increases in color after standing one hour.

Much work has also been done upon the effects of different conditions such as temperature, effects of salts, acids, and bases, and varying amounts of the reducing reagent. Chapman (6) used the rotating color disc and showed that the temperature affects the rate and amount of color produced by the Deniges method. Truog and Meyers (7) using the Deniges method have shown that ferric iron in greater concentrations than six parts per million depresses the development of blue color and also gives a greenish tint. However, the reduction to the ferrous condition eliminates this trouble. They also suggest this test can be used for a quantitative test for phosphates in the presence of

arsenates, if both are present in a sample. The two may first be determined together. In another sample the arsenic may be reduced with hydrogen sulfide or a little sodium sulfide in acid solution. After the excess sulfide is removed by boiling and filtered, the phosphorus may be determined and the concentrations may be determined by difference.

Different methods have been used to prepare the extract for the determination of phosphorus. Different methods give different information regarding the availability or solubility of the inorganic phosphorus.

Dyer (23) used a one per cent citric acid solution, Das (10) used a one per cent potassium carbonate solution, Fraps used a .2 N nitric acid solution, while Perkins (21), King, and Benne used distilled water as a means of preparing the sample.

There has been much discussion in the literature about whether organic phosphorus can be utilized by plants. In a recent publication, Pierre and Parker (17) have shown that plants do not take up organic phosphorus, but only inorganic phosphorus.

THE SPECTROPHOTOMETER

The instrument used is a Bausch and Lomb Spectrophotometer. It consists essentially of three units: (1) a

means of illumination of the sample, (2) a rapid means of varying and comparing light intensities, (3) a spectrometer which divides the transmitted beam into its constituent colored components. The light source is a concentrated filament 250 watt lamp. The light from this source is passed through a double ground glass window. The beams which are then parallel are reflected by two right angle prisms so that they will pass through plungers and vertical specimen holders. The plungers and specimen holders are of the colorimeter type. The plungers are hollow and fixed in position. The holders are mounted on a movable base which can be accurately controlled to one-tenth millimeter depth of solution by a vernier scale. The paths of the parallel beams after passing through the solution are altered again by two right angle prisms and are then focused upon the apertures of the photometer.

The photometer is of the polarization type in which the two halves of the field are polarized at right angles to each other by means of a Wollaston prism. Variation of intensity of light between the two halves is made by the rotation of a prism of the Glan-Thompson type, the rotation of which is read on a circular scale divided in degrees. Light first enters the photometer at a distance of 40 millimeters apart. After leaving the Wollaston prism, the ordinary ray from one beam converges with the extraordinary

ray from the other beam into the Glan-Thompson prism and are brought back parallel to each other by a bi-prism. Due to the difference in effect of the inclined surface of the bi-prism on the polarized beam, the amount of light transmitted by the two halves of the bi-prism is not the same and consequently the zero point of the instrument is not exactly at 45° . The method employed in calculating absorption is independent of the match point so that an exact setting at 45° is unnecessary. In order to avoid difficulties due to polarization in the sample, such as strains in glass or liquid polarization, reading of absorption is taken in one position and the photometer is rotated 180° about the optical axis, securely clamped, and read again.

The two beams from the photometer pass into the spectrometer and the spectra of the two are spread out in close juxtaposition before the eye. The prism of the spectrometer is mounted on a table which may be rotated by a lug on the prism table and is operated by a knurled head outside the drum chamber. The distinctive feature of this instrument is that the telescope and collimator are fixed, and that observations can be made through the entire spectrum by rotating a drum head on which is read the wave length of the portion of the spectrum which is being investigated.

METHODS AND PROCEDURE

Method of Deniges

The procedure recommended for the Deniges method of determining phosphorus is as follows (4) (7):

"Reagent A -- Mix 100 cubic centimeters 10 per cent ammonium molybdate solution with 300 cubic centimeters of a 50 per cent by volume solution of sulfuric acid (arsenic free). The reagent should be kept in the dark or in a brown bottle.

"Reagent B -- To .5 grams of powdered tin, add 5 drops of a 4 per cent solution of Cu SO_4 and 10 cubic centimeters of concentrated hydrochloric acid (arsenic free). Warm to hasten the reaction, and when the tin is in solution, dilute to 50 cubic centimeters. This reagent must be prepared fresh each day.

"Standard P_2O_5 solution -- Dissolve .1917 grams of KH_2PO_4 in distilled water, transfer quantitatively to a 1000 cubic centimeter volumetric flask and dilute to mark. This solution contains 100 P. P. M. P_2O_5 . Take 10 cubic centimeters of this solution and dilute to 1000 cubic centimeters in a volumetric flask to obtain a solution of 1 P. P. M. P_2O_5 . In like manner 20 cubic centimeters made up to 1000 cubic centimeters gave 2 P. P. M. P_2O_5 , etc.

To prepare a solution containing 1 P. P. M. P_2O_5 , dilute 50 cubic centimeters of solution that contains two parts per million to 95 cubic centimeters, add 2 cubic centimeters of reagent A and 6 drops of reagent B, while shaking, and dilute to 100 cubic centimeters."

The above directions were followed. The temperature of the room was between 24° - $27^{\circ}C$. The time between adding reagent B and taking reading was between 3 - 15 minutes. This was timed very closely and if this time elapsed before the readings had been taken for one curve or determinations, a new solution was made.

The Molybdenum Blue Method

Preparation of Molybdenum Blue Reagent (2) -- "In a porcelain casserole put 120 cubic centimeters of sulfuric acid (density 1.78), free from arsenic, add 6.02 grams of powdered molybdenum trioxide. This is then boiled and stirred until all of the MoO_3 is dissolved. It is cooled and made up to 200 cubic centimeters with distilled water. This is solution I.

"To 100 cubic centimeters of solution I add .2800 grams of newly powdered molybdenum metal and heat to boiling, stir constantly to avoid bumping. After allowing it to cool, decant off the liquid into a graduated test glass and complete to 100 cubic centimeters with distilled water.

This is solution II.

"The reducing power of the final concentration of Mo O_2 should be determined against KMnO_4 . 2.51 cubic centimeters of final reagent should decolorize .2 cubic centimeters N KMnO_4 . In order to get a solution to have the reducing power desired, we standardize solution II.

"For this standardization, we take exactly .2 cubic centimeters of N KMnO_4 and add drop by drop of solution II until the color disappears. On calculating how much of solution I to add to solution II for .2 cubic centimeters of N KMnO_4 to exactly decolorize 2.51 cubic centimeters of the final solution. It is this solution that we call the reagent of Molybdenum Blue. This reagent has a blue color but it loses its color when it is diluted or put in the unknown. If the solution contains phosphorus, arsenates, or more than 6 milligrams of SiO_2 per liter, the blue will occur again.

Technique of Method -- "Put 50 cubic centimeters of unknown or known sample into a beaker and add the reagent. The amount of reagent depends upon the amount of phosphorus in solution. If solution contains between .05- .1 milligram of phosphorus in 50 cubic centimeters, add exactly .3 cubic centimeters of the reagent. If the solution contains more phosphorus (.1 - .5 mg.) add exactly .6 cubic centimeters of reagent. Boil this solution for 4 - 5

minutes and allow to cool, make back to 50 cubic centimeters.

This solution when stoppered and kept in the dark retains the same color intensity for 7 - 10 days. When kept in light, a decrease in color intensity is noticed after 2 or 3 days."

The above directions were followed and a few corrections were necessary in order to get results that could be duplicated. The time of boiling was increased to 15 minutes before maximum color was reached. Continued boiling did not affect color intensity.

When this method was tried out on some soil that contained yellow organic material and boiled for 15 minutes, there was a little precipitate which was necessary to be filtered off. This was filtered and washed with distilled water and made back up to volume and the color intensity was determined.

Preparation of Soil Sample

An acid loam soil from Cherokee County, Kansas, was selected for use in this experiment. A sample of the air dried soil was carefully crushed, then screened through a 20-mesh sieve to remove the rocks and coarse organic matter. Enough of this soil was treated to give several 100-gram samples. The soil was treated by adding about two times as much $\text{N}/10 \text{ KCl}$ as soil sample by weight. This sample was

thoroughly shaken, allowed to settle, and the supernate liquid was decanted off. This was repeated until all of the calcium had been replaced as was shown by test for calcium on the supernate liquid. Nineteen washings were required before this was accomplished. The soil sample was then washed first with water and later with alcohol until all of the chloride ions were washed out. The sample was allowed to dry. This is the same procedure followed by Perkins, King, and Benne (21).

One-hundred grams of this soil were taken and 250 cubic centimeters of distilled water was added. The water had been aerated by bubbling air through it for four hours. The soil solution was shaken on a continuous shaker for four hours and then centrifuged. Fifty cubic centimeters aliquot portion of this solution was taken and forced through a Pasteur-Chamberland filter. The clay filter had previously been ignited to red heat, allowed to cool, and washed with 50 cubic centimeters $N/10$ HCl followed by water to remove the acid. After the soil extract had been passed through the filters, the soil particles were rinsed from them with distilled water using a brush. Forty-five cubic centimeters $N/10$ HCl was run through the filters and this added to the soil extract.

Enough $NaOH$ to neutralize the acid was added in a 5 cubic centimeter portion. The molybdenum blue reagent was

added and boiled for 15 minutes. It was filtered to remove settled organic material and made up to 100 cubic centimeters. A portion of the soil solution was taken that did not have the molybdenum blue reagent added, but the same amount of acid was added. This solution was used as the solvent mentioned previously.

The Spectrophotometer Measurements

The spectrophotometer was placed before a mercury arc light and the ocular slit adjusted so that the reading shown by the wave-length drum corresponding to the accepted value for the mercury lines. The error of the wave-length drum was not more than .3 $m\mu$ for any line. This setting was also checked using sodium and lithium flames. The absorption curve of Amaranth dye was taken following the directions given (24) by the Scientific Paper of Bureau of Standard. The maximum absorption was found to be at 520.8 $m\mu$.

The collimator slit is controlled by a micrometer screw with a head reading to .1 millimeter. According to Fechner's law, the minimal difference which can be distinguished in the intensity of adjacent fields is proportional to the total intensity and is about 1 to 2 per cent under ideal conditions. Therefore, the collimator slit was varied so that the observation field was always at minimal

brightness in order to be able to detect slight difference in intensity. The eye piece of the spectrophotometer was kept narrow so as to limit the region of the spectrum observed to approximately 3 - 5 m μ .

One cell was filled with the solution to be observed. A similar cell was filled with the solvent to correct for any light scattered or absorption due to the cell and solvent. The spectrophotometer was adjusted and the two solutions were placed at the desired position in the paths of the two light beams. The wave-length drum was set for the portion of the spectrum to be observed and the analysis prism of the photometer was rotated until a match in the comparison field was observed. The total intensity of the field was adjusted by the collimator slit. Record was made of the degrees on the scale on the drum that controlled the analyzing prism. The wave-length was changed and similar readings were taken until the range of the visible spectrum had been covered. The photometer was reversed and the entire spectrum was read as before.

The usual formula given for the Marten's type photometer is:

$$T = \frac{I_0}{I} = \tan^2 \theta .$$

where I and I_0 have the same significance as already stated and θ the angle of rotation of the analyzer prism. The

above formula assumes a match point at 45° , and also assumes that the substance is optically inactive. One of the advantages of the Marten type of photometer is that the effects of any polarization due to the specimen and of any deviation of the match point from 45° is completely compensated by reversing the photometer. If θ_1 is the scale reading in the second quadrant and θ_2 is the scale reading in the first quadrant then

$$T = \frac{\tan \theta_2}{\tan \theta_1} = \tan \theta \times \cot \theta_1.$$

$$\text{or } -\log T = \log \tan \theta_1 \text{ plus } \log \cot \theta_2.$$

The best match could be obtained when θ_1 was close to 75° or where θ_2 was close to 15° . This could be controlled by varying the depth of the solution.

EXPERIMENTAL OBSERVATIONS

A soil extract was made up from a sample of soil, as has already been explained. An absorption curve of the soil extract has been given in figure 1. Water was used in the solvent cup. The other curve on figure 1 represents the absorption curve of the yellow soil extract to which was added enough phosphorus to make the concentration two parts per million P_2O_5 plus the phosphorus extracted from the soil sample. Four-tenths cubic centimeters of molybdenum blue

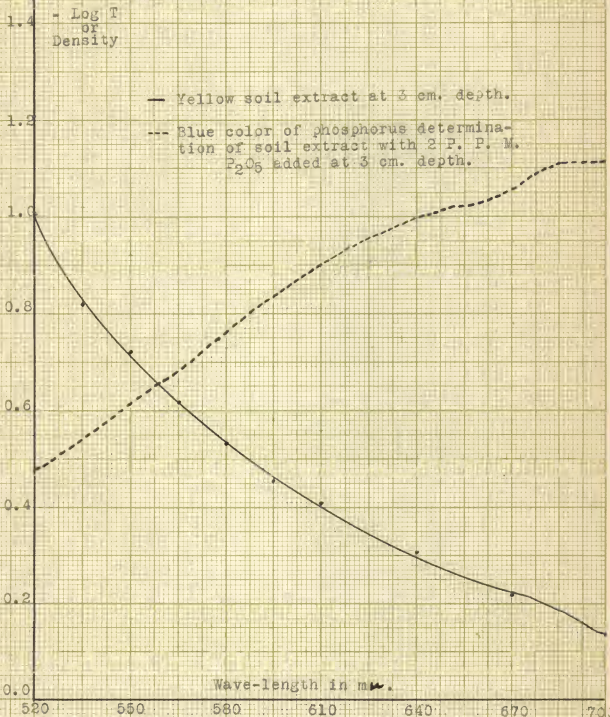


Fig. 1. Showing relation between absorption curve of yellow soil extract and blue color of phosphorus determination.

reagent was added to a 50 cubic centimeter portion while only acid was added to another 50 cubic centimeter portion. Both solutions were boiled for 15 minutes and made up to 50 cubic centimeters. The yellow soil extract was used in the solvent cup. The absorption curve of the blue color developed is shown on one of the absorption curves in figure 1.

Figure 2 shows the relation between the absorption curves of the two methods investigated. The color was developed upon a solution containing four parts per million P_2O_5 . The directions for developing both methods were followed. The solution in the solvent cup was distilled water in both methods, because no noticeable amount of light was absorbed by either reagent when in a water solution of the concentration required to develop the color.

Figure 3 shows the absorption curves of the blue color produced by the molybdenum blue method for various concentrations of phosphorus, the depth remaining constant in each case. In this case, larger amounts of the molybdenum blue reagent were used when more phosphorus was present. When one part per million of phosphorus was present 0.4 cubic centimeters of molybdenum blue reagent was used with an increase of 0.1 cubic centimeter for each additional part per million.

- Log T
or
Density

1.4

— Molybdenum blue method 4 P. P. M.
at 3 cm. depth.

--- Deniges method 4 P. P. M. at 1.5
cm. depth.

1.2

1.0

.8

.6

.4

.2

Wave-length in mμ.

460

500

540

580

620

660

700

Fig. 2. Showing relation of absorption curves of
the molybdenum blue method and the
Deniges method.

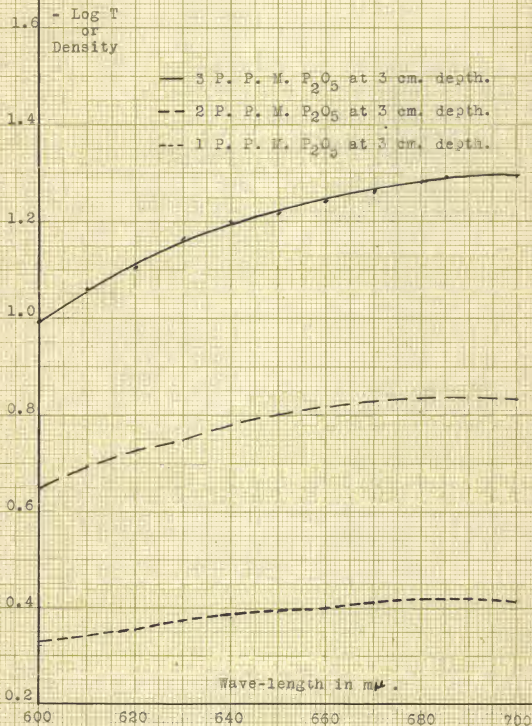


Fig. 3. Absorption curves of the molybdenum blue method.

Figure 4 shows the absorption curves of the blue color produced by the Deniges method using aliquot of the same phosphorus solutions that were used in the determinations used in figure 3.

After finding the curve for maximum absorption, it was necessary to find the extinction coefficient at this wavelength. The extinction coefficient was found by substituting the factors in the following formula: $\epsilon = \frac{-\log T}{cd}$.

Where ϵ is the extinction coefficient, c is concentration of phosphorus in parts per million of P_2O_5 , d is depth in centimeters, and $-\log T$ is found from the rotation of the analyzing prism.

The blue color was developed by the molybdenum blue method on the yellow soil extract as mentioned above, but no phosphorus was added. The value for $-\log T$ at five centimeters depth was found to be .52942 which gives 0.73 parts per million P_2O_5 . When two parts per million P_2O_5 was added to the yellow extract, the curve of which is shown in figure 1, $-\log T$ was found to be 1.1145 at 3 centimeters depth. This figure gives 2.75 parts per million P_2O_5 , a difference of only .031 parts per million less than the original sample. The incident light in both cases was sent through an acid portion of the yellow soil extract.

Yellow extracts from soils other than that which was

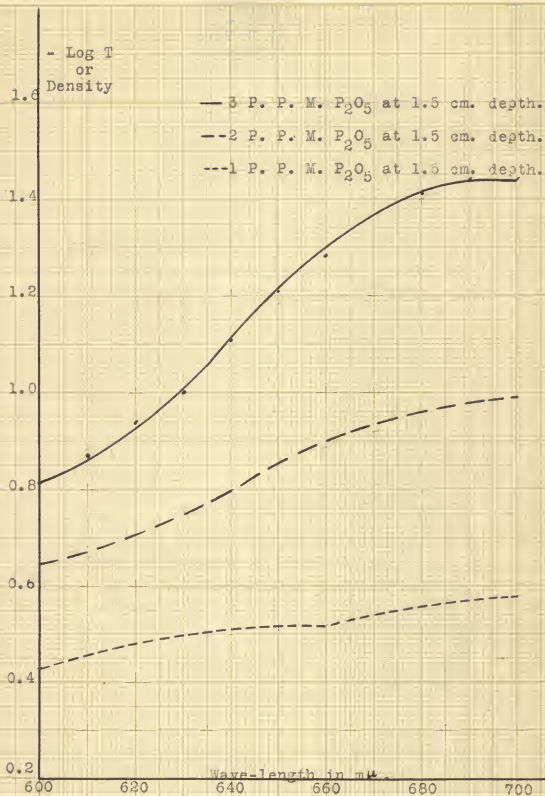


Fig. 4. Absorption curves of the Deniges method.

saturated with potassium absorbed light in the same region of the spectrum and yield absorption curves similar to the one described above.

The following table gives results on the molybdenum blue method following directions already given and using a wave-length of $685 \text{ m}\mu$.

Table 1. Determination of the Extinction Coefficient for Molybdenum Blue Method

P.P.M. P_2O_5	Depth in: centi- meters	c.c. of: reagent	θ , in degrees	θ_2 in degrees	$-\log T$	ϵ
1	5	.4	64.2	25.0	.64701	.130
2	3	.5	68.2	19.8	.8369	.140
2	4	.5	73.5	14.2	1.125	.140
2	5	.5	76.7	11.4	1.3218	.132
3	3	.6	75.1	12.5	1.2293	.136
4	2	.7	73.2	14.6	1.1138	.139
Average						.136

The extinction coefficient was determined on the color produced by the Deniges method, using aliquot of the same phosphorus solutions used above and the same wave-length. The following table shows the results from which the average value was obtained.

Table 2. Determination of the Extinction Coefficient
for Deniges Method

P.P.M. P_2O_5	Depth in centi- meters	θ_1 in degrees	θ_2 in degrees	$-\log T$	ϵ
1	4	76.1	11.0	1.31836	.329
2	1.5	70.4	16.8	.96856	.336
3	1	74.2	15.4	1.00822	.336
3	1.5	77.6	9.6	1.4342	.318
4	1	74.8	12.2	1.231	.308
Average					.322

The effect of ferric iron upon the molybdenum blue method was investigated using varying amounts, weighed as ferric chloride. The ferric chloride solution was made up and aliquot portions were added to the phosphorus solutions so that each sample contained 2 P. P. M. P_2O_5 and the amount of iron indicated in the table. Four-tenths cubic centimeters of the molybdenum blue reagent was used to develop the color. A wave-length of 685 $m\mu$ was used.

The following table shows how increasing amounts of ferric ion decreased the color intensity.

Table 3. Effects of Ferric Iron upon the Molybdenum
Blue Method

P. P. M. Fe as Fe Cl ₃	Depth in centi- meters	-log T	P. P.M. P O 2 5 found
0	3	.82755	2.02
2	4	1.1075	2.04
4	4	1.1142	2.05
6	4	1.1010	2.03
8	4	1.1243	2.06
10	4	1.1171	2.05
20	4	1.1075	2.03
50	4	.7817	1.43
100	No blue color produced only a yellow color		

The following experiment was conducted to determine if it were possible to wash clay filters with N/10 H Cl and neutralize this acid with sodium hydroxide without affecting the intensity of color developed. Samples were prepared so that when they were brought to a final volume of 50 cubic centimeters they would contain 2 P. P. M. P₂O₅ and the concentration of base and acid indicated in table 4.

Table 4. Effects of Neutralizing Hydrochloric Acid
with Sodium Hydroxide on the
Molybdenum Blue Method

Depth in centi- meters	Normality of acid	Normality of base	-log T	P. P. W. P_2O_5
5	.025	----	.43391	.64
5	.01	----	.75529	1.11
4	.00	.00	1.06393	1.95
4	--	.01	1.03807	1.91

Experiments were run to determine the effects of different acids upon the blue color developed by the molybdenum blue method. Samples were prepared containing different amounts of acids as indicated below. Each sample after being made to 50 cubic centimeters contained 2 P. P. W. P_2O_5 . Four-tenths cubic centimeter of molybdenum blue reagent was used to develop the color.

Table 5. Effects of Concentration of Acids and Bases on Molybdenum Blue Method

Acid or base used	Depth in centi- meters	Normal- ity	pH after color was developed	-log T	P. P. M. P ₂ O ₅
-----	4	----	1.94	1.115	2.04
H ₂ SO ₄	4	.025	1.95	1.103	2.02
H ₂ SO ₄	5	.1	1.90	.13719	.20
Citric:	4	.025	1.94	1.1142	2.04
Citric:	4	.1	1.93	.86243	1.58
Lactic:	4	.025	1.94	1.01068	1.85
Lactic:	--	.1	1.93	-----	-----*
Acetic:	4	.025	1.94	1.1142	2.04
Acetic:	5	.1	1.93	.43391	.63
HCl	5	.025	1.95	1.34799	1.98
HCl	--	.1	1.90	-----	-----*
Na OH	4	.025	1.95	1.16926	2.14
Na OH	--	.1	9.21	-----	-----**

* In the cases where the solutions were very acid, the color did not develop at all.

** In the cases where the solutions were very basic, the color developed, but faded within two or three minutes.

Note: The pH values in the above table were determined by the quinhydrone method.

DISCUSSION

The general belief is that only inorganic phosphorus is available for plant food. The results of Pierre and Parker (17) uphold this assumption. So investigations were restricted only to the determination of inorganic phosphorus.

Figure 1 shows that the yellow color of extract of soil which had been treated to replace all replaceable ions with K^+ does not absorb light in the same region as the blue color of the phosphorus determination. The other curve in figure 1 shows the absorption of light after the blue color was developed in the soil extract containing plus 2 P. P. M. of additional P_2O_5 . The incident light was passed through an acid extract of the yellow soil extract.

Figure 2 shows that the color intensity produced by the Deniges method is very much more intense than that produced by the molybdenum blue method on a sample that contained the same amount of phosphorus. Both reached maximum absorption at $685\text{ m}\mu$. Teorell (3) has reported that he found maximum absorption at $720\text{ m}\mu$. This is about the limit of the detection of light with the eye. The light was very hard to match above $690\text{ m}\mu$. so it may be possible that the blue color has greater absorbing powers

higher in the spectrum, however, this would have to be detected by some other method than the human eye.

Figure 3 shows that the curves for the molybdenum blue method are uniform at different concentrations and different amounts of phosphorus. On the other hand, figure 4 shows that the curves for different amounts of phosphorus for the Deniges method are irregular.

Work has been done by Truog and Meyers (7) on the effects of ferric iron and other salts upon the intensity of the blue color produced by Deniges' method. They have shown that ferric iron interferes with this method when in concentration greater than 6 parts per million. Chapman (8) has shown how the temperature and how ferrous iron interferes with the development of the blue color. Chapman has also shown how varying amounts of the reagent affect the intensity of the color.

Table 3 shows the effects of different acids and sodium hydroxide upon the development of the blue color by the molybdenum blue method. The pH values of the solutions, measured after the color was developed, are recorded here. The acids which are highly dissociated caused a much greater decrease in color than those that are weakly dissociated. Low concentrations of sodium hydroxide seemed to increase the color intensity. This was found to be true by Truog (7) when the Deniges method is used.

The reactions of the solutions after the color is developed show that the molybdenum blue reagent acts as a buffer holding the pH near 1.94. If the pH is greater than this, more basic, the color develops and then fades. If the pH is lower, more acid, the color does not develop at all.

Ferric iron in amounts of 20 parts per million does not interfere with the color intensity when developed by the molybdenum blue method.

The molybdenum blue reagent was kept in the laboratory for 9 months without any change. The color developed on phosphorus solutions and remained at the same intensity for 36 hours. Zinzadze reports that the color will not fade for two to three days in the light or seven to eight days in the dark.

The intensity of color of the Deniges method will not remain constant for more than 15 minutes, as reported by Chapman (8) in the same concentrations of reagents as used in this thesis. The stannous chloride, reagent B, of the Deniges method must be made up fresh each day, which is a disadvantage of this method.

The color develops on the Deniges method within one minute as reported by Chapman (8) at room temperature, while the molybdenum blue method required boiling for 15

minutes. The boiling has some effect at times causing the yellow organic substance to precipitate. The organic material may react with the reagent also. More of the reducing agent may be added to eliminate this objection. When the organic substance precipitates, it is necessary to filter it off. Osmond (1) reports that a thin layer of asbestos is best for use as a filter as it does not absorb the blue color like paper does.

The extinction coefficient for the blue color of the molybdenum blue method is 0.156 where one part per million of P_2O_5 is dissolved in distilled water using one centimeter depth at a wave-length of $685m\mu$. The extinction coefficient of the Deniges method is 0.322 using the same notation as used above. This shows that the Deniges method gives more than twice as much color as the molybdenum blue method.

The limits that one can use the spectrophotometer by the Deniges method are between 0.5 to 4 parts per million P_2O_5 , while that of the molybdenum method are from 1 to 5 parts per million P_2O_5 . Since it is necessary to boil the solution, anyway, in the molybdenum method the solution may be concentrated by boiling and made up to a smaller volume. This enables one to determine smaller concentrations of phosphorus.

Much work has been done upon the relation of the concentration of inorganic phosphorus in soil to plant growth. Parker (8) has reported that corn can grow in a solution as low as .1 P. P. M. PO_4 , but maximum growth of corn is reached in solutions containing .4 P. P. M. PO_4 . Tidmore (19) reports maximum growth of corn in solutions that contain .25 P. P. M. PO_4 . Both grew plants in a culture solution.

SUMMARY

1. The spectrophotometer can be used to determine inorganic phosphorus in soil extracts.

2. Maximum absorption for both the molybdenum blue method and the Deniges method is reached at a wave-length of 685 m μ .

3. The yellow color of soil extract, that is, where exchange complex is saturated with potassium, absorbs light in the blue portion of the spectrum and absorbs little light in the red region where the blue phospho-molybdate compounds absorb. By using the soil extract made up to the same acid concentration as the sample, that is being tested, and using this to send the incident light through. The color effects of the yellow color can be eliminated.

4. The extinction coefficient for the blue color of

the molybdenum blue method is 0.136 where one part per million of P_2O_5 is dissolved in distilled water using one centimeter depth at a wave-length of 685 m μ . While the extinction coefficient for the blue color of the Deniges method is 0.322 using same solution and conditions as was used in the molybdenum blue method.

5. According to the law of Beer the compounds that give the blue color in both determinations are not the same.

6. The pH of the solution of the molybdenum blue method must be 1.94 before maximum color will develop. The molybdenum blue reagent acts as a buffer and will buffer a concentration of .025 N acid or base.

7. Ferric ions in concentration less than 20 parts per million will not interfere with the intensity of the blue color produced by the molybdenum blue method.

8. The color produced by the Deniges method fades in 15 minutes, while the color produced by the molybdenum blue method stays constant for at least 36 hours.

9. The absorption curves for the molybdenum blue method are even and agree nicely at different wave-lengths, while the absorption curves for the Deniges method show some irregularity.

10. The limits that one can use the spectrophotometer

with the Deniges method are between 0.5 to 4 parts per million P_2O_5 with an accuracy of about 10 per cent. The limits with the molybdenum blue method are between 1 to 5 parts per million P_2O_5 with an accuracy of about 4 1/2 per cent.

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