PHOSPHORUS ANALYSIS OF PLANT MATERIAL[1]

B. R. BERTRAMSON

Introduction

Although satisfactory methods for the determination of total phosphorus in plant material are available, they are, for the most part, too long and complicated to serve where a great number of comparative tests in studies of phosphorus nutrition of plants are desired. These methods provide for either volumetric or gravimetric determinations. For the former, 2 to 4 gm. of plant material are required; for the latter, 6 to 12 gm. of material are necessary. The amounts of available material are frequently less than these. By means of a colorimetric method used by the author in phosphorus studies, duplicate samples of only 0.25 gm. were found to check quite consistently within the range of 1 or 2 per cent. in routine analyses. This method is similar to the official micro-method in the 1940 edition of "Official and Tentative Methods of Analysis" (**2**) but is somewhat less time consuming.

It is the purpose of this paper to report upon a method for the determination of total phosphorus and the inorganic fraction of phosphorus in plant material.

Total phosphorus

ASHTON (**1**), and WEISSFLOG and MENGDEHL (**14**) proved that ashing of plant material was equally as satisfactory as wet combustion for successful results. ASHTON found that where 2 m.e. of $Mg(NO_3)_2$ were added per gm. of dry plant material, ashing could be carried on at a temperature of 800° C. without loss of phosphorus.

One of the objections to ashing has been the contention that the pyro- and metaphosphates resulting from the high temperatures of ashing are converted with difficulty to orthophosphate. MENGDEHL (**8**) proved, however, that ten minutes of boiling the above salts in N HCl was adequate to convert them to orthophosphate. This was so successful that he devised a method of analysis of pyro- and metaphosphate on this basis.

In an attempt to verify the above findings, 2.5-mg. samples of phosphorus in solution as orthophosphate were placed in evaporating dishes, 2 or 3 m.e. of $Mg(NO_3)_2$ were added, and the solution was evaporated to dryness. Following ignition at 600° to 700° C. for 30 minutes, the ash was dissolved in 10, 15, or 20 ml. of 2 N H_2SO_4, after which 15 ml. of distilled water was added. This was reduced to 5 ml., or less, by evaporation on the steam bath.

[1] Technical Paper no. 389, published with the approval of the Director of the Oregon Agricultural Experiment Station.

The sides of the dish were then washed down and the volume again reduced to 5 ml. on the steam bath. After cooling, the solutions were made up to a definite volume. The results of a series of such tests compared to a standard made up from the same stock solution and in the same manner but omitting the ashing and digesting procedure are summarized in table I. The deter-

TABLE I

RECOVERY OF PHOSPHORUS FOLLOWING ASHING WITH $Mg(NO_3)_2$ AND BOILING WITH H_2SO_4

SAMPLE NO.	TREATMENT		PHOSPHORUS RECOVERED*	RECOVERY†
	$Mg(NO_3)_2$	H_2SO_4		
	m.e.	*m.e.*	*mg.*	%
1	2	20	2.49	99.6
2	2	30	2.48	99.2
3	3	30	2.50	100.0
4	3	40	2.50	100.0

* The phosphorus ashed in each case was 2.50 mg.
† 2.5 mg. = 100 per cent.

minations were made according to the modified TRUOG and MEYER method as described by BERTRAMSON (3), using a KLETT-SUMMERSON photoelectric colorimeter. It was demonstrated in a previous publication (3) that the amount of $MgSO_4$ present in these tests as a result of the subsequent neutralization of the MgO with H_2SO_4 could be 30-fold greater without affecting the test appreciably. From these studies the following procedure was developed and found very satisfactory for routine analysis.

PROCEDURE

Weigh duplicate samples of finely ground and well mixed plant material which has been dried at 60° C. for 48 hours. (The size of sample will be determined largely by the uniformity and amount of material at hand. A 0.5-gm. sample was generally found most convenient where the material had been ground fine enough to pass a 20-mesh screen, or finer, and had been thoroughly mixed. However, 0.1-gm. samples of properly prepared material are adequate.) Place the material in evaporating dishes of approximately 100- to 150-ml. capacity. If the sample used is 0.5 gm., or less, add 5 ml. of 0.5 N $Mg(NO_3)_2$ and 10 to 15 ml. of distilled water. Evaporate to dryness on a steam bath. Remove and allow the dish to dry before placing in the hot muffle furnace. Ignite at 600° to 700° C. until the residue is uniformly gray in color; 30 minutes is usually sufficient time. Remove from the muffle, cover with a watch glass, and allow to cool until liquid can be added without spattering. Then add 15 ml. of 2 N H_2SO_4 and revolve the dish so that the acid comes into contact with all of the ash. Add 15 to

20 ml. of distilled water and place the dish on the steam bath. Evaporate to a volume of less than 5 ml., wash down the sides of the dish with 10 to 15 ml. of distilled water, and again reduce the volume to about 5 ml. (It is doubtful whether the concentration of silica will in any case interfere. If it does, it may be removed at this point by the usual method of dehydration and subsequently taking up the phosphorus in an acid solution.) Remove the dish from the steam bath and add 15 to 25 ml. of distilled water. After the contents have cooled to room temperature, rub down the sides of the dish with a rubber policeman and transfer the contents quantitatively to a 100-ml. volumetric flask, make to volume, and store in a stoppered Erlenmeyer flask until aliquots have been analyzed colorimetrically by the modified TRUOG and MEYER method.

Inorganic phosphorus

It is often desirable to know the amount of inorganic phosphorus in various plant materials. Likewise, if one knows the total phosphorus content of plant material and can determine the inorganic fraction, it is possible to calculate, by difference, the phosphorus fraction that is organically combined. WEISSFLOG and MENGDEHL (**14**) worked out and tested methods for the separation of the total phosphorus of fresh plant material into three fractions—namely, inorganic, soluble organic, and insoluble organic. DE TURK (**5**) made one separation on the basis of solubility in alcohol as well as those mentioned above. EMMERT (**6**) also developed a method for the determination of inorganic phosphorus of fresh plant material, using 1 per cent. by volume H_2SO_4 as the extracting solution. Where fresh material is used for analysis, the processes of obtaining a representative sample and of storing the material for a period of time are complicated. The practice of decolorizing the extracts with charcoal or by other means has been rendered unnecessary by the introduction of the photoelectric colorimeter in which the color values of the extracts may be deducted from the test reading.

It was found that by treating the plant material prepared as for total analysis with a preliminary wetting agent, such as methyl, ethyl, or n-butyl alcohol, the material assumed physical properties very similar to those of fresh plant material and the EMMERT method (**6**) could be applied. Upon investigating the effect of time of extraction and of various treatments during extraction upon the plant material, however, it was found that the 5-minute period of extraction suggested by EMMERT was not sufficient to obtain a constant value. The various methods of treatment are summarized in table II. From these studies it is evident that in the case of finely ground and dried material the triturating or application of the mechanical shaker in extraction is unnecessary. Furthermore, the use of a wetting agent as

mentioned above is superfluous with finely ground and dried tomato plants. (For some materials such a preliminary treatment might be advantageous.) The values obtained after periods of extraction from 30 minutes to 17 hours were found to agree satisfactorily whereas values for less than 30 minutes of extraction decreased slightly as the period of extraction was decreased. The data indicate that extraction with the 1 per cent. sulphuric acid solution as suggested by EMMERT for fresh material is equally applicable to the finely ground and dried material if the time of extraction is extended to 30 minutes.

Satisfactory recovery of phosphorus added to the extracts has been shown, using charcoal to eliminate the extraneous color (**6, 14**). This was checked by the author using the photoelectric colorimeter and omitting the decolorizing treatment. The recovery was found fully satisfactory, indicating that the extraneous organic material did not affect this colorimetric test.

TABLE II

EFFECT OF TIME AND TREATMENTS UPON THE EXTRACTION OF PHOSPHORUS FROM DRIED AND GROUND PLANT MATERIAL

SAMPLE NO.	TREATMENT WITH EXTRACTION	PHOSPHORUS OBTAINED AT VARIOUS TIME INTERVALS OF EXTRACTION					
		5 MIN.	15 MIN.	30 MIN.	60 MIN.	2 HR.	17 HR.
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
68	(1) Wetting agent*; triturated in mortar	1,342	1,450	1,460	1,510	.....	.....
68	(2) In Erlenmeyer; wetting agent; shaken occasionally	1,363	1,442	1,455	1,455	.....	.....
68	(3) Same as (2) except in mechanical shaker	1,400	1,442	1,480	1,480	.....	.....
68	(4) Same as (2) except H_2O as wetting agent	1,442	.....	.....	1,510	.....	.....
88	Same as (1) then placed in stoppered Erlenmeyer	612	.....	.....	.....	673	660
92	Same as for no. 88	506	.....	.....	.....	562	562

* The wetting agent used was absolute methanol.

The stability of organic forms of phosphorus in plant material was demonstrated by MENGDEHL (**8**) when he found that boiling plant material for 15 minutes with N HCl did not bring about appreciable hydrolysis. In order to determine the stability of the filtered extracts, aliquots of several of the extracts from various samples of tomato plants were analyzed for phosphorus immediately following extractions of 5 and 60 minutes and the remainder of the extracts was kept in tightly stoppered Erlenmeyer flasks for periods of 3 to 10 days at room temperature. The results of this study are summarized in table III. The acidity of the extracts was such as to

preclude the activity of microorganisms and increases in inorganic phosphorus of the extracts upon standing for several days could be wholly attributed to hydrolysis of the organic phosphorus; however, only slight increases in inorganic phosphorus were found. This was more noticeable in the 5-minute extractions and indicated that this short period of extraction was of insufficient duration for equilibrium to be established. The stability of the organically combined phosphorus under the above conditions identified it as a definite fraction of the phosphorus contained in plants.

TABLE III

STABILITY OF PHOSPHORUS EXTRACTIONS FROM PLANT MATERIAL

Sample no.	Phosphorus determined in extracts			Percentage increase in P in 3 days	Percentage increase in P in 10 days
	Immediately after 5-min. extraction	3 days after 5-min. extraction	10 days after 5-min. extraction		
	p.p.m.	*p.p.m.*	*p.p.m.*	%	%
16	536	555	.....	3.5	.....
20	714	724	.....	1.4	.....
24	622	642	.....	3.2	.....
28	444	449	.....	1.1	.....
Same time periods as above but with 60-minute extraction					
68	1,490	1,494	1,494	0.3	0.3
96	1,144	1,156	1,174	1.0	2.6

Phosphorus determined in the above manner may be considered as the inorganic fraction, sharply differentiated from the organic fraction. In view of the reproducible results and the foregoing studies regarding treatment and extractions, the following method is proposed for the direct determination of the inorganic phosphorus of plant material and the indirect determination of the organic phosphorus fraction.

Procedure

Weigh out into Erlenmeyer flasks 0.5-gm. samples of finely ground and well mixed plant material which has been dried at 60° C. Add by means of a pipette 50 ml. of a 1 per cent. by volume solution of sulphuric acid. Stopper the flasks and shake vigorously several times during the next 30 minutes. Filter through acid-washed (arsenic-free) filter paper and store the filtrate in stoppered Erlenmeyer flasks. Aliquots of the filtrate may then be analyzed colorimetrically by the modified Truog and Meyer method (**3**).

The modified Truog and Meyer colorimetric method is, briefly, as follows: Pipette an aliquot of the solution, containing 0.002 to 0.07 mg. of phosphorus as orthophosphate into a 50-ml. volumetric flask. Add a drop or two of 0.5 per cent. solution of para-nitrophenol indicator. Bring the volume to approximately 25 ml. with distilled water, add 1:1 NH_4OH drop-

wise until a yellow color appears; then back titrate dropwise with 2 N H_2SO_4 until the color just disappears. Add 2 ml. of 2.5 per cent. ammonium molybdate in 10 N H_2SO_4 until the color just disappears. Make up to volume, mix well, and place in a stoppered Erlenmeyer flask. When a series of solutions have been prepared, add to each from a dropper (**3**) 0.23 ml. of 1 per cent. stannous chloride solution of at least 90 per cent. stannous tin. Whether the readings are made by direct comparison or on a photoelectric colorimeter, the tests should be allowed to stand for the same period of time following the addition of stannous chloride as in the case of the standards. The 15- to 20-minute period of standing is recommended.

Effect of ferric iron

The effect of ferric iron upon the color test has been reported by several workers (**4, 11, 12**). Smith *et al.* (**11**) pointed out that 1 p.p.m. of ferric iron in a solution containing 0.2 p.p.m. of phosphorus exerted a negligible

TABLE IV

Effect of ferric iron on the modified Truog and Meyer colorimetric test*

Concentration of P	Percentage error at various concentrations of ferric iron					
	None	2 p.p.m.	4 p.p.m.	8 p.p.m.	16 p.p.m.	32 p.p.m.
p.p.m.	%	%	%	%	%	%
0.1	0.0	3.1	4.2	10.4	.....	.....
0.5	0.0	2.9	.....	.....	.....	.....
1.0	0.0	3.8	5.0	5.5	6.8	47.5

* Tests were made at 23° C. and readings were made 15 to 20 minutes after the addition of $SnCl_2$.

effect but at higher concentrations the errors were appreciable. Their conclusion was that dilution of the solution to the lower limits of the test was the best practical means of avoiding trouble where ferric iron was present. A brief survey of the literature regarding the relative iron and phosphorus content of a wide range of plant material (**9, 13**) indicates that this practice would be applicable in all cases and with but few exceptions the phosphorus content greatly exceeds the iron content of plants thereby eliminating the trouble entirely.

In table IV are summarized the percentage errors resulting from the presence of various amounts of ferric iron at different concentrations of phosphorus. The error with concentrations of ferric iron as high as 4 p.p.m., in the form of ferric chloride, appears to be somewhat similar for high and low concentrations of phosphorus. At 2 p.p.m. the error was approximately 3 per cent. for concentrations of phosphorus from 0.1 to 1.0 p.p.m. Thus, plant material with a ferric iron content 20 times greater than the phosphorus content could be handled by this method with an error of 3 per cent.

plus the experimental error. Such a ratio of iron to phosphorus in the plant doubtless never exists. With a ferric iron content only 2 or 3 fold greater than the phosphorus content, which is found only in unusual instances, the iron would not be a serious handicap.

A simple test of any kind of plant material would quickly indicate whether or not ferric iron is a serious factor. Where agreement to BEER's law holds over the whole range of phosphorus concentrations used in analyzing aliquots, the ferric iron concentration is not interfering with the test.

Discussion

The modified TRUOG and MEYER method is sensitive to less than 0.05 p.p.m. of phosphorus and the foregoing studies verify its accuracy. EMMERT (**6**) pointed out that the weakness of the original method was the instability of the stannous chloride solution. Storage of this reagent under hydrogen in a simple apparatus described by BERTRAMSON (**3**) has eliminated this criticism. The conformity of the test to BEER's law from 0.05 to 1.75 p.p.m. of phosphorus makes the method especially applicable to routine analysis where a photoelectric colorimeter is used. Lower range concentrations may be analyzed satisfactorily by means of a visual colorimeter.

PARKER and FUDGE (**10**) pointed out that the FISKE and SUBBAROW method (**7**) was satisfactory for analysis of solutions containing more than 1 p.p.m. of phosphorus. The modified TRUOG and MEYER method is sensitive to less than 0.05 p.p.m. of phosphorus. Hence, the advantage of the former in being unaffected by 30 p.p.m. of ferric iron is largely nullified by the fact that the same solution could be analyzed by the modified TRUOG and MEYER method simply by dilution to such an extent that the ferric iron concentration would not interfere.

The speed and accuracy of the method make it especially desirable for comparative studies in phosphorus nutrition experiments. Furthermore, by a determination of total and inorganic phosphorus of plant material, the organic phosphorus fraction may be calculated as the difference between the above values. A method whereby the total, inorganic, and organic phosphorus of plant materials may be quickly and accurately determined should be especially useful in working with the complex problems of phosphorus metabolism and interrelated problems in plant nutrition.

Conclusions

1. A method is presented whereby the total and inorganic phosphorus portion of plant material may be determined quickly and accurately.

2. Data are submitted to prove the stability of the organic phosphorus in a one per cent. by volume sulphuric acid solution; thereby demonstrating that it is a definite fraction of the total phosphorus in plant material.

The writer is greatly indebted to DR. R. E. STEPHENSON of the Soils

Department for criticisms and suggestions during the preparation of the manuscript.

OREGON STATE COLLEGE
CORVALLIS, OREGON

LITERATURE CITED

1. ASHTON, F. L. Influence of the temperature of ashing on the accuracy of the determination of phosphorus in grass. Jour. Soc. Chem. Ind. **55**: 106T–108T. 1936.
2. ASSOC. OFFIC. AGR. CHEM. Official and tentative methods of analysis. 5th ed. Washington, D. C. 1940.
3. BERTRAMSON, B. R. Studies on the ceruleomolybdate determination of phosphorus. Soil Sci. **53**: 135–141. 1942.
4. CHAPMAN, H. D. Studies on the blue colorimetric method for the determination of phosphorus. Soil Sci. **33**: 125–134. 1932.
5. DETURK, E. E. Chemical transformations of phosphorus in the growing corn plant, with results on two first-generation crosses. Jour. Agr. Res. **46**: 121–141. 1933.
6. EMMERT, E. M. A method for the rapid determination of phosphate in fresh plant tissues. Plant Physiol. **5**: 413–417. 1930.
7. FISKE, C. H., and SUBBAROW, Y. The colorimetric determination of phosphorus. Jour. Biol. Chem. **66**: 375–400. 1925.
8. MENGDEHL, H. Studien zum Phosphorstoffwechsel in der höheren Pflanze. I. Die Bestimmung von Pyro- und Metaphosphate, sowie von Phosphit und Hypophosphit in Pflanzenmaterial. Planta **19**: 154–169. 1933.
9. MITCHELL, J. H., WARNER, J. D., and MORROW, K. S. The mineral content of feeds, soils, and waters of South Carolina. South Carolina Agr. Exp. Sta. Bull. 252. 1928.
10. PARKER, F. W., and FUDGE, J. F. Soil phosphorus studies. I. The colorimetric determination of organic and inorganic phosphorus in soil extracts and the soil solution. Soil Sci. **24**: 109–117. 1927.
11. SMITH, G. W., DYER, T. J., WRENSHALL, C. L., and DELONG, W. A. Further observations on the determination of phosphate by photoelectric colorimetry. Canadian Jour. Res. B **17**: 178–191. 1939.
12. TRUOG, E., and MEYER, A. H. Improvements in the Deniges colorimetric method for phosphorus and arsenic. Ind. Eng. Chem. Anal. Ed. **1**: 136–139. 1929.
13. VANSLYKE, LUCIUS L. Fertilizers and crops. Orange Judd Pub. Co., New York. 1927.
14. WEISSFLOG, J., and MENGDEHL, H. Studien zum Phosphorstoffwechsel in der höheren Pflanze. III. Aufnahme und Verwertbarkeit organischer Phosphorsäureverbindungen durch die Pflanze. Planta **19**: 182–241. 1933.