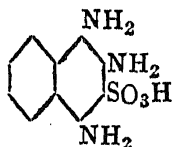# IMPROVEMENTS IN COLORIMETRIC DETERMINATIONS

I. Phosphates-Correlation Between Total and Dilute Acid Soluble Phosphate in Non-calcareous Soils

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I. INTRODUCTION

THE age old Colorimetric method of determining phosphates by Denige (1920) has undergone considerable modifications. The method originally consisted in the addition of a reducing agent stannous chloride to a solution of phosphates to which had been added previously a solution of ammonium molybdate and sulphuric acid. A blue compound named phospho-conjugated ceruleo-molybdate which has a formula according to Denige $[(M_0O_3)_4 M_0O_2] H_3PO_4, 4H_2O$ is formed. The colour intensity of this compound in solution is directly proportional to the concentration of phosphates. Fiske and Subbarow (1925) used $1:2:4$ aminonaphthol sulphonic

acid ⌬ (NH₂, NH₂, SO₃H, NH₂) as reducing agent. They applied for the first time an

organic reducing agent in the ceruleo molybdate reaction. Parker and Fudge (1927) compared the methods of Denige, and Fiske and Subbarow with special reference to reducing agents and concluded that stannous chloride used by Denige was five times more efficacious than $1:2:4$ aminonaphthol sulphonic acid advocated by Fiske and Subbarow. Truog and Meyer (1929) found that the excess of hydrochloric acid and ferric iron in more than 6 p.p.m. interfered with the reaction, while neither titanium at concentration lower than 20 p.p.m., nor nitrates and salts of aluminium, manganese, calcium and magnesium did so. They, therefore, advised to evaporate the hydrochloric acid and reduce the ferric iron to ferrous condition. Chapman (1931, 1932) standardised the colour intensity on Helige comparator and made standard colour discs for quick and precise estimations. He also devised methods of preserving stannous chloride solution

from atmospheric oxidation in aspirator bottles in which the top surface of the solution is covered with one inch thick layer of paraffin or some other light mineral oil. Warren and Pugh (1930) further modified the method so that it might have application to hydrochloric and citric acid extracts. They oxidised the organic matter by sodium permanganate, precipitated the iron and manganese as ferrocyanides at a pH of 6·8 to 6·9 and determined the phosphate in the filtrate by the usual Denige's method. They were first in the field to determine colour intensity by Klett and Kober colorimeter which was a more accurate method than the comparison in Helige Comparator by Chapman. Hance (1936) used hydrochloric acid and ammonium molybdate with stannous chloride as reducing agent for estimating phosphates in soil extracts. He made permanent colour standards in glass vials and compared the colour in phosphate illuminator designed by him. Dyer and Wrenshall (1938) used photo-electric colorimeter for measuring colour intensity and studied the inhibiting effects of a series of mono and dibasic organic acids on colour development, besides also the kinetics of the reactions using stannous chloride as reducing agent. They concluded that organic acids have an inhibiting effect on the colour reaction and the maximum colour is developed five minutes after the addition of 2·5 per cent. solution of stannous chloride. They also showed that in solutions of lower phosphate content the maximum intensity is maintained for five minutes or more but fading begins rather rapidly at higher concentrations thereby showing that the blue compound is highly labile at such concentrations. Morgan (1937) used solid stannous Oxalate in the rapid method of estimating phosphate in soil extracts, but the kinetics of the reactions with the reducing agent was not studied. The use of stannous oxalate as reducing agent in solid condition is preferred over stannous chloride solution as the latter is easily oxidised in atmosphere and consequently necessitates storage under paraffin oil.

In the work reported hereunder, an attempt has been made (1) to study the kinetics of stannous oxalate in solid condition with a view to determine the minimum quantity of the substance required to develop maximum colour intensity in the shortest possible time limit, Klett Sommerson Photo-electric colorimeter with green filter ($500\,\mu\mu$ to $570\,\mu\mu$) being used for the purpose; (2) with the standard method thus developed, to determine the maximum extraction of phosphates by mineral acids, on a number of soi samples drawn from different situations in North Bihar; and (3) to correlate total phosphates with the mineral and organic acid soluble phosphates in the non-calcareous soil types.

II. EXPERIMENTAL

(1) *Kinetics of Stannous Oxalate*—The procedure followed in these experiments was to measure colour intensity by means of photo-electric colorimeter in aliquotes of the standard phosphate solutions by addition of different quantities of stannous oxalate (solid) at different time intervals. The usual Truog's solution was used before addition of stannous oxalate for developing the colour.

(*a*) *Minimum quantity of stannous oxalate (solid) for maximum colour intensity.*—Six concentrations of standard phosphate solutions were tried against six doses of stannous oxalate ranging from 0·001 gm. to 0·11 gm. A solution of 50 p.p.m. of phosphorus was made by first dissolving 0·2195 gms. of potassium dihydrogen phosphate in water and diluting it to 1 litre. 50 c.c. of this solution were diluted to 500 c.c. to give the desired stock reagent (5 p.p.m. of phosphorus). Aliquotes of this stock solution were taken and diluted to 95 c.c. with water, 4 c.c. of Truog's reagent (ammonium molybdate and sulphuric acid) being added to each of the solutions, which were finally diluted to 100 c.c. Stannous Oxalate was added to the solutions in solid condition in different doses and the scale of the colorimeter was read at the point of maximum deflection. The results (Table I) show that the colour intensity goes on increasing with the increase in the quantity of stannous oxalate added till a constant is reached between 0·06 and 0·1 gm., the quantity actually required (Fig. 1) being approximately 0·08 gm. for the maximum intensity of colour.

TABLE I

Showing quantity of stannous oxalate and colour intensity

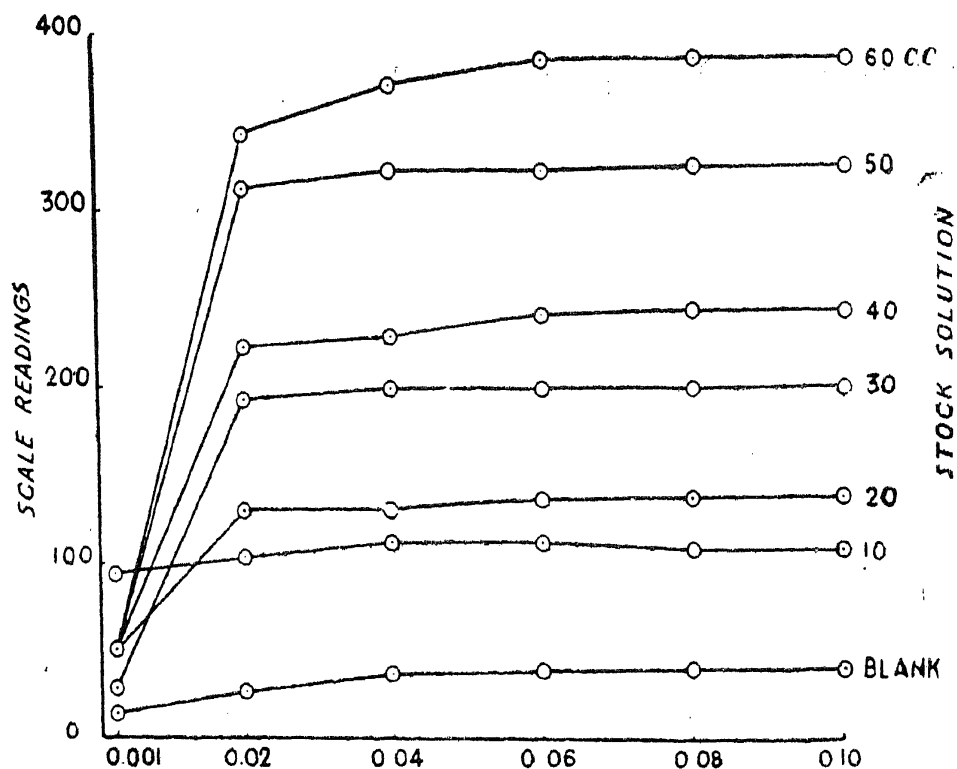| Stannous oxalate gm.	Vol. of solution stock	0 cc.	10 cc.	20 cc.	30 cc.	40 cc.	50 cc.	60 cc.
	Equivalent P p.p.m.	Blank	0·5	1·0	1·5	2·0	2·5	3·0
0·001	..	13·5	93·0	50·0	49·5	26·5	49·0	53·0
0·021	..	25·5	107·5	127·0	191·0	219·0	310·0	340·0
0·041	..	36·5	110·5	129·0	198·0	221·0	320·0	369·0
0·061	..	39·0	110·5	135·0	198·9	239·0	320·0	383·0
0·081	..	40·0	109·5	136·0	198·0	243·0	325·0	385·0
0·110	..	40·0	110·0	137·5	196·5	243·0	323·0	385·0

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Fig. 1. Quantity of Stannous Oxalate in gms.

(b) *Minimum time for maximum colour intensity.*—The intensity of the colour developed in standard solutions according to the above method was measured at even intervals between two to twenty minutes. The results (Table II, Fig. 2) show that the colour intensity attains a maximum value within 8–10 minutes after the addition of stannous oxalate. On keeping

TABLE II

Showing time and maximum colour intensity

| Time in minutes	Vol. of stock solution	0 cc.	5 cc.	10 cc.	20 bc.	30 cc.	40 cc.	50 cc.	60 cc.
	Equiva-lent P p.p.m.	Blank	0·25	0·50	1·0	1·5	2·0	2·5	3·0
2	..	20·0	64·0	120·5	214·0	283·0	327·5	397·5	512·5
4	..	21·0	71·0	122·5	217·0	305·0	355·0	417·5	555·0
6	..	27·5	77·5	131·0	221·0	310·0	365·0	432·5	565·0
8	..	30·0	86·0	134·5	231·0	320·0	370·0	437·5	557·5
10	..	30·0	86·0	135·5	231·0	322·5	375·0	450·0	595·0
12	..	30·0	90·5	135·5	230·0	322·5	375·0	452·5	595·0
14	..	35·0	91·5	139·0	239·0	337·5	387·5	450·0	595·0
16	..	36·5	99·5	142·5	249·0	342·0	397·5	465·0	595·0
18	..	40·0	103·0	145·0	252·0	342·0	400·0	482·5	612·5
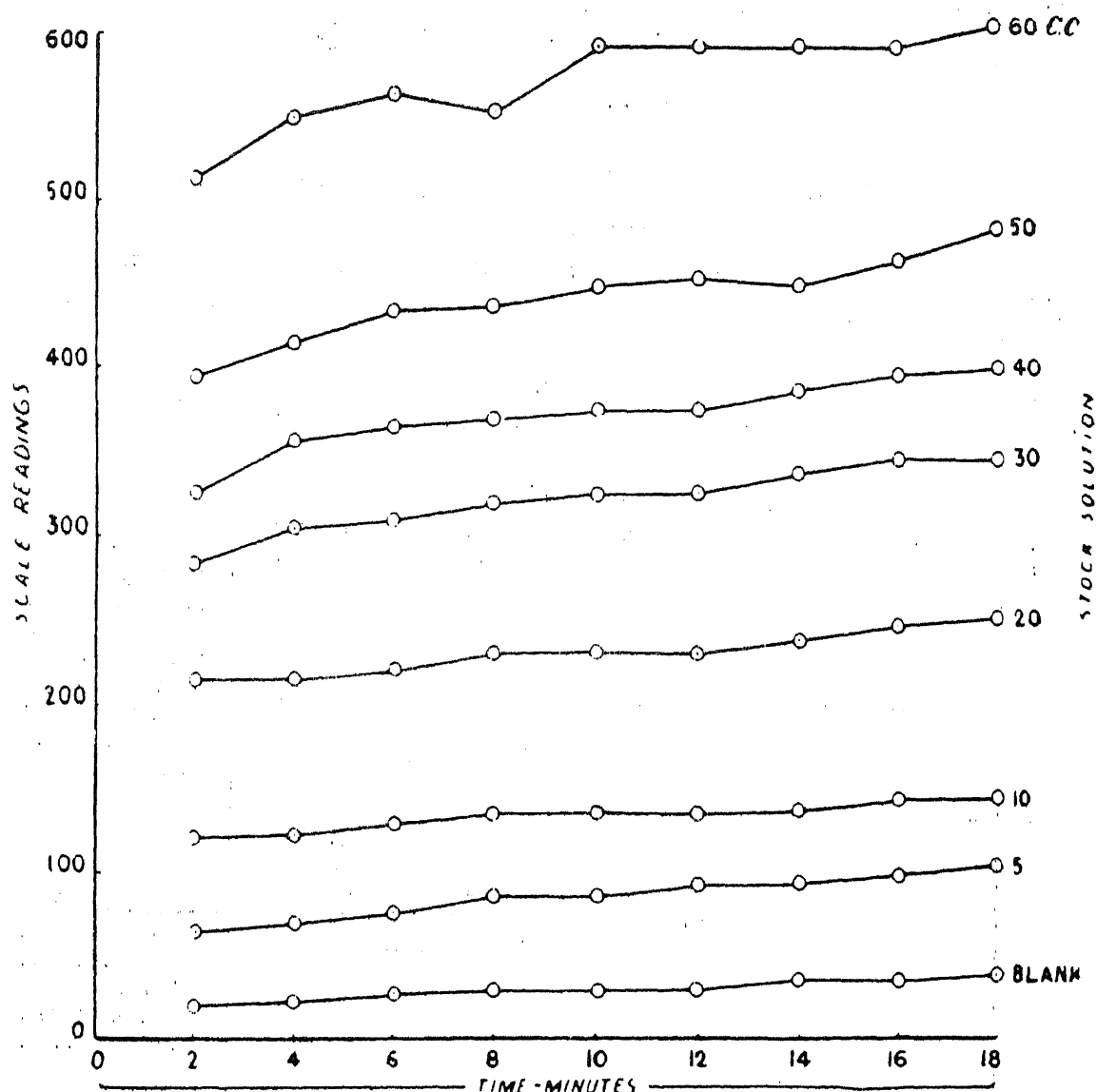
FIG. 2. Time and Colour Intensity

the solution for a longer period, a sharp rise in the scale reading of the colorimeter was observed due to the decomposition and precipitation of the highly labile phospho-molybdate complex when the clear blue solution loses the transparency required in colorimetric measurements and becomes turbid. Experiments repeated during May-June and December-January periods at Pusa gave the same results and it may, therefore, be concluded that the complex is not thermo-labile at least for this period (10 minutes) within the range of variations of the laboratory temperatures (25° C.-35° C.) at Pusa. The time of observation of colour intensity was, therefore, fixed at approximately 10 minutes after the addition of stannous oxalate.

(2) *Maximum extraction of Phosphates by mineral acids.*—The extraction of phosphates by acids and other extracts is a complicated problem. The quantity extracted differs with different soils besides depending upon factors like the quantity of soil, volume and strength of the extracting solution,

etc. Soil workers have, therefore, used different extracting solutions to suit different conditions in different countries (Table III).

TABLE III

Showing methods of testing available phosphates in soils

Sl. No.	Year and place where originated	Author	Extracting Reagent	Remarks
1	Deubeny	1845, England	Carbonic acid	
2	Von Liebig	1872, Germany	Dilute hydrochloric or acetic acid	
3	Lechartier	1884, France	2 percent ammonium oxalate	
4	Dyer	1894, England	1 percent citric acid	
5	A. O. A. C.	1907, U. S. A.	0·2 normal hydrochloric acid	
6	Das	1926, India	Alkaline corbonate solutions	For calcareous soils pot. and sod. corbonates better than ammon. carbonates
7	Truog and Mayer	1929, U. S. A.	0·002 normal sulphuric acid buffered with ammonium sulphate at pH 3·0	Am. molybdate + sulphuric acid and stannous chloride
8	Arrhenius	1929, Java	1 per cent sodium chloride solution containing enough sulphuric acid to make the normality 0·002N	
9	Thornton	1931, Indiana	0·1 per cent normal hydrochloric acid solution	Am. molybdate + hydrochloric acid with stannous chloride
10	Morgan	1937. Connecticut U. S. A.	Sodium acetate and acetic acid 5 normal pH 4·8	Sodium molybdate and stannous oxalate
11	Spurway	Michigan, U. S. A.	Dilute acetic acid about 3·2 pH	
12	Bray	Illionis, U. S. A.	0·1 normal hydrochloric acid solution	
13	Fraps	Texas, U. S. A.	0·2 normal nitric acid	Denige's method
14	Hester	Virginia Truck, U. S. A.	Sodium acetate and acetic acid at pH 5·0	do
15	Harper	Oklahome, U.S.A.	0·2 normal sulphuric acid	
16	Hance	1937. Honolulu, Hawaii	0·5 normal hydrochloric acid	Thornton's method
17	Dirks and Scheffer	Germany	1 gm. calcium carbonate in 250 cc. water and then saturating with carbondi-oxide	
18	Von Drangell	Germany	Water	
19	Limmerman	1923, 1926, Germany	Ten per cent hydrochloric acid and one per cent citric acid	
20	Lohse and Ruhnke	England	Potassium bi-sulphate	
21	Dalburg and Brown	U. S. A.	Sod. acetate 0·25 M and acetic 0·08 N	Sod. molybdate + stannous oxalate
22	Hockernsmith, Gardner and Godwin	Indiana	Potassium carbonate one per cent	

A perusal of Table III will show that it is chiefly the mineral acids that have been frequently used and attempt was, therefore, made to determine a quantity of dilute mineral acid (HCl) that would give the maximum extraction of phosphates. For this purpose, four soil types markedly differing in calcium and phosphate contents were taken. One gramme of soil from each of these four samples was shaken with different volumes (4 c.c. to 50 c.c.) of the N.HCl acid, filtered after the effervescence had subsided and phosphate determined colorimetrically in the filterate by using Thornton's reagent (ammonium molybdate and hydrochloric acid) and stannous oxalate as reducing agent. The results (Table IV, Fig. 3) show that maximum extraction is afforded by 1 gm. of soil and 20 c.c. of normal hydrochloric acid.

TABLE IV

Showing extraction of phosphates by hydrochloric acid

Analysis of HCl extract			pH of extracting soln.	5 cc. H₂O		4 cc. N·HCl		8 cc. N·HCl		12 cc. N·HCl		16 cc. N·HCl		20 cc. N·H Cl		50 cc. N·HCl	
Sl. No.	CaO%	P₂O₅%	Below	pH	P p.p.m.	pH	P p.p.m.	pH	P p.p.m.	pH	P p.p.m.	pH	P p.p.m.	pH	P p.p.m.	pH	P p.p.m.
1	0·862	0·1582	4·0	7·5	nil	4·0	3·0	4·0	4·1	4·0	4·4	4·0	4·8	4·0	5·2	4·0	5·4
2	11·49	0·0441	4·0	7·0	nil	7·0	0·17	4·0	1·5	4·0	2·2	4·0	2·4	4·0	2·4	4·0	2·4
3	21·88	0·0719	4·0	8·0	nil	7·0	nil	6·0	0·19	5·0	0·98	4·0	1·5	4·0	1·5	4·0	1·5
4	31·63	0·0486	4·0	..	nil	8·0	nil	7·5	nil	5·0	0·24	4·0	1·5	4·0	1·5	4·0	1·47

(3) *Correlation between available (mineral and organic acid) and total phosphate in non-calcareous soils.*—Using the method as finally standardised in the earlier sections of this paper, about one hundred samples collected from different factory reserved areas of North Bihar were analysed for total and acid soluble phosphates and total calcium for finding out the magnitude of correlation. The analysis of total phosphates was done by Pemberton's (1893, 1894) volumetric method and that of total calcium by the usual oxalate method. The organic (acetic) acid soluble phosphates were determined by Morgan's method (*loc. cit.*). The procedure for the modified method of determining mineral acid phosphate was as follows:

(i) *Reagents.—(a) Truog's Reagent.*—Dissolve 25 gms. of Ammonium molybdate in 20 c.c. of water, heat to 60° C., dilute 280 c.c. of conc. sulphuric acid to 800 c.c. When both solutions have cooled down, add the Ammonium molybdate solution to that of the sulphuric acid with shaking.
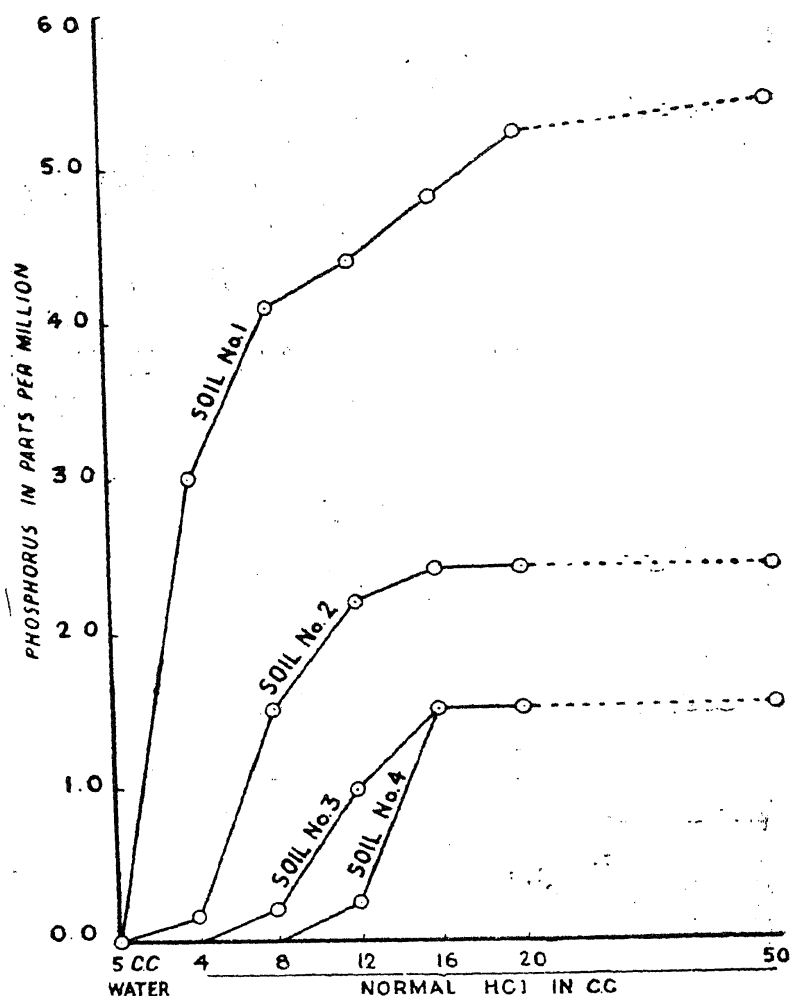
FIG. 3. Extraction of Phosphates

(b) *Stannous oxalate.*—0·08 gm. of stannous oxalate, in solid condition.

(c) *Soil extract.*—N.HCl.

(ii) *Extraction and estimation of phosphates.*—Treat 1 gm. of soil with 20 c.c. N.HCl, stir with glass rod till effervescence subsides, filter; add 10 drops of conc. nitric acid and evaporate to dryness on hot plate, but do not allow it to bake; add 10 drops of conc. HCl and 10 drops of conc. HNO_3 and again evaporate to dryness; further add 4 c.c. of Truog's reagent (Ammo. molybdate + H_2SO_4) and transfer to 100 c.c. flask; make up the volume and add stannous oxalate (0·08 gm.) in the solid state; mix well and compare in colorimeter with a standard solution of phosphate after standing for ten minutes.

The results (Table V) show the correspondence that exists between the values obtained by the different methods.

TABLE V

Showing estimation of phosphates by different methods

Sl. No.	Sample No.	Locality	Total $P_2O_5\%$ (Y)	Truog's $P_2O_5\%$ (X)	Av. calculated P_2O_5 $Y = 0.0344 + 2.5492\,X$	Morgan's Phosphate value in lb/ac.	Total $CaO\%$
1	663/100·S	Hassanpur R.A.	0·11	0·04	0·14	25	1·9
2	664/101·S		0·13	0·04	0·14	10	1·2
3	665/102·S		0·10	0·06	0·18	10	0·13
4	667/104·S		0·10	0·09	0·09	25	1·4
5	668/105·S		0·10	0·04	0·14	50	2·0
6	669/106·S		0·10	0·04	0·14	50	1·3
7	670/107·S		0·10	0·04	0·14	25	1·9
8	671/108·S		0·10	0·04	0·14	50	1·5
9	672/109·S		0·05	0·03	0·08	50	1·9
0	673/110·S		0·11	0·04	0·14	50	1·5
11	674/111·S		0·11	0·03	0·11	25	1·2
12	680/117·S		0·11	0·04	0·14	50	1·0
13	681/118·S		0·11	0·04	0·14	50	1·1
14	682/119·S		6·12	0·05	0·16	50	0·9
15	683/120·S		0·07	0·05	0·16	50	1·3
16	686/123·S		0·09	0·03	0·11	50	1·06
17	687/124·S		0·10	0·04	0·14	50	0·79
18	688/125·S		0·10	0·03	0·11	50	0·64
19	689/126·S		0·10	0·05	0·16	50	1·48
20	690/127·S		0·12	0·04	0·14	50	0·79
21	291/128·S		0·09	0·06	0·19	50	1·12
22	265/29·S	Sakri R.A.	0·07	0·01	0·06	50	0·36
23	266/30·S		0·02	0·01	0·06	100	0·39
24	267/31·S		0·04	0·005	0·05	25	0·35
25	268/32·S		0·58	0·09	0·26	10	1·5
26	269/33·S		0·57	0·12	0·44	10	1·5
27	270/34·S	"	0·06	0·006	0·05	50–100	0·6
28	271/35·S		0·05	0·003	0·04	10	0·3
29	272/30·S		0·09	0·02	0·08	25	0·8
30	273/37·S		0·52	0·12	0·44	25	0·8
31	274/38·S		0·42	0·05	0·16	50	0·90
32	275/39·S		0·07	0·02	0·08	25–50	0·5
33	276/40·S		0·07	0·02	0·08	50–100	0·5
34	277/41·S		0·10	0·06	0·18	25–50	0·4
35	278/42·S		0·04	0·005	0·05	10	0·4
36	279/43·S		0·23	0·13	0·36	50	0·6
37	280/44·S		0·42	0·12	0·34	25	1·2
38	281/45·S		6·11	0·04	0·13	10	0·5
39	282/46·S		0·09	0·04	0·13	100	0·3
40	283/47·S		0·05	0·006	0·05	50	0·3
41	284/48·S		0·59	0·06	0·18	25–30	0·8
42	285/49·S	Harinagar R.A.	0·12	0·08	0·24	25	0·62
43	286/50·S		0·06	0·005	0·05	10	0·59
44	287/51·S		0·15	0·024	0·10	25	0·29
45	288/52·S		0·06	0·003	0·04	25–50	0·35
46	289/534S		0·04	0·008	0·05	50	0·31
47	290/54·S		0·18	0·06	0·18	25	0·55
48	291/55·S		0·21	0·05	0·04	25	0·55
49	292/56·S		0·21	0·08	0·23	10	0·63
50	293/57·S		0·04	3·004	0·04	50	0·46
51	294/58·S		0·08	0·03	0·11	25	0·69
52	295/59·S		0·13	0·00	0·19	50–100	1·09

TABLE V—(*Continued*)

Sl. No.	Sample No.	Locality	Total $P_2O_5\%$ (Y)	Truog's P_2O_5 (X)	Av. calculated $P_2O_5\%$ Y=0·0344+ 2·5492 X	Morgan's phosphate value in lb/ac.	Total CaO%
53	296/60·S	Haringar R.A.	0·19	0·05	0·16	50	0·53
54	297/61·S		0·04	0·007	0·05	10	0·53
55	298/62·S		0·22	0·004	0·04	50	0·40
56	299/63·S		0·04	0·012	0·06	100	0·38
57	300/64·S		0·19	0·009	0·06	25	0·49
58	301/65·S	Lohat R.A.	0·53	0·12	0·34	50	0·18
59	302/66·S		0·16	0·07	0·21	50'	0·62
60	303/67·S		0·28	0·11	0·31	10	0·67
61	305/69·S		0·17	0·04	0·14	25	0·57
62	306/70·S		0·15	0·06	0·19	10	0·73
63	307/71·S		0·06	0·004	0·04	25	0·33
64	308/72·S		0·13	0·06	0·19	40	1·0
65	309/73·S		0·08	0·01	0·06	15	0·60
66	310/74·S		0·04	0·03	0·11	15	0·50
67	312/76·S		0·10	0·02	0·09	25	0·87
68	314/78·S		0·07	0·01	0·06	10	0·84
69	315/79·S		0·26	0·12	0·34	25	0·70
70	316/80·S		0·05	0·005	0·05	25	0·53
71	319/83·S		2·22	0·10	0·29	80	0·59
72	320/84·S		0·07	0·003	0·04	50	0·56
73	322/86·S		0·08	0·002	0·09	60	0·30
74	325/89·S		0·05	0·005	0·05	60	0·42
75	328/92·S		0·05	0·004	0·04	10	0·42
76	329/93·S		0·06	0·003	0·04	10	0·37
77	330/94·S		0·09	0·03	0·11	15	0·64
78	141/25·Y	Narkatiaganj R.A.	0·07	0·01	0·06	10	0·29
79	142/26·Y		0·10	0·04	0·14	10	0·29
80	143/27·Y		0·12	0·04	0·14	25	0·38
81	144/28·Y		0·06	0·01	0·06	10	0·39
82	145/29·Y		0·16	0·07	0·21	25	0·73
83	146/30·Y		0·09	0·04	0·13	10	0·38
84	147/31·Y		0·05	0·002	0·04	10	0·43
85	148/32·Y		0·06	0·005	0·05	10	0·29
86	149/33·Y		0·09	0·04	0·13	25	0·35
87	150/34·Y		0·05	0·01	0·08	10	0·42
88	151/35·Y		0·13	0·07	0·21	25	0·28
89	152/36·Y		0·09	0·03	0·11	25	0·33
90	153/37·Y		0·05	0·001	0·04	10	0·69
91	154/38·Y		0·04	0·006	0·05	10	0·38
92	155/39·Y		0·07	0·006	0·05	10	0·59
93	156/40·Y		0·125	0·05	0·16	25	0·46
94	157/41·Y		0·08	0·04	0·13	25	0·46
95	158/42·Y		0·11	0·04	0·14	25	0·50
96	159/43·Y		0·07	0·02	0·09	10	0·39
97	160/44·Y		0·04	0·005	0·05	10	0·49
98	161/45·Y		0·06	0·02	0·09	10	0036
99	162/46·Y		0·04	0·009	0·06	10	0·39
100	163/47·Y		0·10	0·04	0·14	10	0·53
101	164/48·Y		0·05	0·006	0·05	10	0·38

The above values of total and acid soluble phosphates were taken for calculating the correlation coefficient between them. The value of 'r' between the total and mineral (N.HCl) soluble phosphate is $+ 0.7098$ and

this is highly significant. The regression equation taking 'Y' as total P_2O_5 and 'X' as mineral acid soluble P_2O_5 is $Y = 0\cdot0344 + 2\cdot5492 \, X$. The values thus obtained appear in column six of the table. The error of estimation for the 101 observations has been calculated to be $0\cdot0079$, which is evidently low. The correlation of the total with the organic acid soluble phosphate (Morgan's R.C.M. test) is exceptionally low being only $-0\cdot168$. It may, therefore, be concluded that the mineral acid method is superior to that of organic acid in the extraction of total phosphates in non-calcareous soils and this may be applied with advantage for their rapid evaluation.

III. Summary

Attempt has been made to study in detail the present procedure being followed in the colorimetric estimation of phosphates and to suggest in the first series of the papers, improvement in the technique so far as it relates to such determinations in non-calcareous soil types.

1. The maximum colour intensity in Ceruleo-molybdate reaction of phosphate estimation in relation to the quantity of reducing agent required and the minimum time necessary for the purpose was determined. The quantity of stannous oxalate necessary was $0\cdot08$ gm. and the time factor 10 minutes. Allowing the solution to stand for longer period resulted in the decomposition and precipitation of the highly labile phospho-molybdate complex. This latter, however, was not thermolabile for this short duration.

2. The correct ratio of soil to extracting acid for maximum phosphate extraction was found to be 1 gm. of soil to 20 c.c. N.HCl.

3. Using the above modifications in Truog's method, about a hundred samples collected from different factory reserved areas in North Bihar, were analysed for total and acid soluble phosphates. The mineral acid extract of soluble phosphates gave a highly significant correlation ($r = +0\cdot7080$) with the total phosphates. The regression values were: $Y = 0\cdot0344 + 2\cdot5492 \, X$ where 'Y' stands for total P_2O_5 and 'X' for mineral acid P_2O_5. For rapid evaluation of phosphates mineral (N.HCl) extract was, therefore, superior to that of organic (acetic) acid.

IV. Acknowledgments

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