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A. B. CONNER, DIRECTOR  
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DIVISION OF CHEMISTRY

## Estimation of Nitric and Nitrous Nitrogen in Soils



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A study of the changes in the nitrogen of the soil involved in the process of nitrification required study of the method for estimating nitrates in soils and cultures. Considerable discrepancies in the amounts of nitric nitrogen as measured by the colorimetric phenol-disulphonic acid method and by the Tiemann-Schulze and reduction methods, led to considerable study of the methods. The differences were found to be due to nitrites in considerable quantities in some of the cultures. Details of the alpha-naphthylamine method for nitrites were modified to render it suitable for estimating nitrites in soils or cultures of soils. The zinc ferrous-sulphate method for nitrates and nitrites was modified for use in testing the methods, the ammonia being finally estimated by titration or by nesslerizing. When allowance was made for nitrites, the phenol-disulphonic acid method was found to be accurate and best suited to work on soils. Various details of the methods were studied. Detailed descriptions of the methods are given.

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## ESTIMATION OF NITRIC AND NITROUS NITROGEN IN SOILS

G. S. FRAPS AND A. J. STERGES

In connection with extensive investigations on nitrification being conducted at the Texas Agricultural Experiment Station, and on account of irregularities, it was found necessary to study the methods for estimating nitrates in soils. As is well known, the phenol-disulphonic acid method occasionally gives unreliable results, due to turbid or discolored solutions, presence of carbonates, chlorides, organic matter, or other factors. Although the method has received considerable study, more work seemed to be necessary, in order to eliminate difficulties as far as possible.

The colorimetric method using phenol-disulphonic acid seemed to give incorrect results on some of the cultures secured in the nitrification tests. Wide differences were found between some of the results by the colorimetric method and by the Tiemann-Schulze method with some cultures of soils, secured in the nitrification work. This cast doubt on the reliability of the colorimetric method and required a study of various details and a search for another method to use in checking the results of the colorimetric method, resulting in modifications of the zinc ferrous-sulphate method. The agreement between the colorimetric method and the zinc ferrous-sulphate method was satisfactory on many samples or cultures, but large differences were found between the two methods on other cultures of soils. These differences were subsequently found (3) due chiefly to the presence of nitrites. On account of the need for a method for nitrites in soils, evident after we found large quantities of nitrites in some cultures of soils, the well known colorimetric method (1) for nitrites was modified to make it better adapted for use on soils. When the nitrites were estimated and allowed for, the results of the colorimetric method, which determines nitrates only, were found to check as well as could be expected with the zinc ferrous-sulphate methods, which include both nitrate and nitrite nitrogen.

Some of the results obtained in the course of the investigation outlined are here presented, together with a detailed description of the methods finally used for the estimation of nitrates and nitrites in soils or cultures of soils secured in nitrification experiments.

Studies of the phenol-disulphonic acid method have been made by Lipman and Sharp (7), Chamot, Pratt and Redfield (2), Harper (5), Van Wijk (10), Whiting, Richmond and Schoonover (11), and others. Comprehensive references to the literature may be found in their papers.

### AMOUNT OF ERROR DUE TO READING OF COLOR IN THE PHENOL-DISULPHONIC ACID METHOD

To ascertain the extent of the variations in the colorimetric method for nitrates, volumes of standard nitrate solution B (1 cc. equals .01 mg. nitrogen) varying from 8 to 25 cc., were measured out by one person and nitrates determined colorimetrically by another. Four series of 12 solutions each were run. The Duboscq colorimeter was used in three series, graduated cylinders in the other. In some cases, there were no differences. The differences found were as follows (see also Table 1): Series 1, —.0 to 3.0, average difference 1.5 cc. (cylinders). Series 2, —1.5 to +.2, average 0.5 cc. Series 3, —6 to 1.8, average difference 0.6 cc. Series 4, = 0.4 to 1.7 cc., average 0.7. Since the amounts taken averaged about 12 cc., the average error with the colorimeter was 4 to 6 per cent of the total, while it was over 10 per cent for the cylinders. Some error must be, of course, expected in reading the color of the solutions. This error depends, to some extent, on the sensitivity of the eyes of the analyst to the graduation of the color used.

Table 1. Error in colorimetric reading for nitrates—Mg. nitric nitrogen

	Colorimeter		Colorimeter		Colorimeter		Cylinders	
	Used Mg.	Found Mg.	Used Mg.	Found Mg.	Used Mg.	Found Mg.	Used Mg.	Found Mg.
	.079	.073	.079	.072	.122	.138	.065	.056
	.118	.122	.182	.180	.087	.094	.073	.080
	.070	.070	.140	.130	.084	.091	.102	.088
	.168	.176	.250	.235	.105	.110	.086	.102
	.081	.082	.095	.099	.135	.142	.105	.100
	.250	.258	.107	.103	.178	.184	.071	.078
	.037	.037	.078	.072	.102	.108	.250	.280
	.130	.143	.100	.098	.082	.089	.169	.196
	.103	.106	.111	.106	.156	.172	.134	.148
	.104	.108	.122	.115	.131	.135	.167	.192
	.228	.240	.090	.086	.085	.091	.101	.120
	.132	.135	.100	.095	.226	.236	.099	.092
Average (12).....	.125	.129	.121	.116	.124	.133	.119	.128
Average + or — dif.....	+.009	-.001	+.003	-.006	+.008	0	+.015	-.009
Standard deviation.....	.008	.....	.007	.....	.009	.....	.017	.....

Another series of experiments was made with additions of the standard solution to quartz sand. Varying quantities of the standard nitrate solution were measured by one person and nitrates determined by the other. Lime was added, nitrates washed out, and the nitric nitrogen estimated by the colorimetric method described in full on a later page. The maximum difference in Series 1 was 1.0 part per million on 7.7 parts per million, or about 12 per cent; the average difference of the 11 was 0.1 on 7.5 parts per million, or a little over one per cent. In Series 2, the maximum difference was 3.3 parts per million on 25 parts per million, or 13 per cent; the average difference of the 10 was 1.5 per million on 28.8, or about 5 per cent, while the standard deviation was 2.7. In Series 3, the maximum difference was 3.5 parts per million on 26.5, or about 14 per cent; the average difference of the

12 was 1.9 parts per million on 43, or about 4 per cent, with a standard deviation of 3.0 for the error. In Series 4 the maximum error was 4.3 parts per million on 34.0, or about 13 per cent; the average error of the 12 was 2.9 parts per million on 43.1, or about 7 per cent, and the standard deviation for the error 3.2 parts per million.

The average error with the colorimetric method is about 5 per cent, while it may run up to 13 per cent occasionally. Thus an error of 20 parts per million may ordinarily occur on a nitrifying culture containing 400 parts per million of nitric nitrogen.

### THE FLOCCULATING REAGENT

As is known, it is sometimes difficult to secure clean filtrates from soils; a good flocculating reagent is needed to aid in securing a clear filtrate. The reagents most commonly used for this purpose are potash alum, alumina cream, carbon black, calcium hydroxide, and copper sulphate. Some of these are satisfactory with some soils and unsatisfactory with others. Lipman and Sharp (7) stated that potash alum had a tendency to give low results, while lime yielded more accurate results.

A comparison was made between lime and potash alum, using soils from cultures incubated for 28 days at 35° C. Some of these cultures had received additions of ammonium sulphate. The lime was used in the modified method as described elsewhere in this paper. The alum was added as 40 cc. of a .5 per cent solution of potassium alum to a sample equivalent to 20 grams of dry soil. After being mixed thoroughly, the liquid was filtered into a 200-cc. volumetric flask, the residue washed, and an aliquot evaporated to dryness. A few drops of concentrated ammonium hydroxide were added to the filtrate before evaporation in order to neutralize any possible acidity which might cause the volatilization of nitric acid during evaporation. The analysis was completed in the usual way. The results of some of the comparisons are given in Table 2.

Table 2. Effect of flocculating reagent on colorimetric nitric nitrogen, in parts per million of soil.

Culture Number	Lime flocculent	Alum flocculent
9.....	287	278
10.....	98	107
11.....	430	435
12.....	104	107
17.....	12	10
18.....	45	40
19.....	320	312
20.....	87	74
21.....	606	606
22.....	116	125
23.....	575	606
24.....	111	118

Both reagents gave nearly the same results. However, potash alum is an unsatisfactory flocculating reagent because the filtrate comes

through very slowly after 4 or 5 washings, and the filtrates from some soils are very turbid. The lower results obtained with alum by Lipman and Sharp were perhaps due to the use of larger amounts of the potash alum than were used in the experiment here described.

Copper sulphate was tried but did not flocculate some of the clayey soils in a satisfactory manner.

Finely ground lime (calcium oxide) was found to be the best flocculent. With some clayey soils, filtration was slow. The filtrates obtained are usually colorless, but occasionally are slightly colored. The colored solution did not affect the results. Some of the filtrates used in the comparison of the phenol-disulphonic acid method and the zinc ferrous-sulphate method, described elsewhere, were slightly colored but no loss of nitrate could be detected.

### EFFECT OF CALCIUM CARBONATE

According to Chamot et al. (2), as well as Harper (5), a mechanical loss of nitrate may take place upon the addition of the phenol-disulphonic acid, to a residue containing carbonates, due to the violent effervescence. When calcium oxide is used as a flocculent, some calcium carbonate remains in the dish after evaporation of the filtrate. The presence of this calcium carbonate might cause loss of nitrates. To test this point, lime was added to measured volumes of standard nitrate solution, the solution was filtered, and the nitrate nitrogen was estimated in one portion of the filtrate. In other portions of the same filtrate the calcium was removed by precipitation with ammonium carbonate and subsequent filtration and nitrates estimated. The excess of ammonium carbonate was volatilized during the subsequent evaporation. In order to eliminate individual bias, the standard potassium nitrate solution (1 cc. = 0.01 mg. N) was measured by one person, and the nitrate determination was made by another, who had no knowledge of the amount of nitrates present. The results are in Table 3.

Table 3. Effect of presence or absence of carbonates on nitric nitrogen by the colorimetric method

Lime present		Lime removed	
Mg. added	Mg. found	Mg. added	Mg. found
.121	.114	.108	.106
.120	.120	.189	.183
.134	.134	.202	.201
.125	.130	.228	.234
.160	.166	.081	.077
.165	.160	.108	.112
.175	.154	.083	.086
.243	.248	.110	.110
.248	.244	.249	.261
.104	.108	.138	.144
.075	.084	.225	.222
.073	.078	.222	.234
Average (12)	.145	.162	.164

The effect of lime was also tested in another experiment. One analyst measured certain amounts of standard potassium nitrate solution into evaporating dishes. Another analyst added 10 cc. of lime water to each dish and estimated nitrates. The results of these tests are given in Table 4.

Table 4. Effect of addition of lime water in the estimation of nitric nitrogen

Dish Number	Mg. added	Mg. found
1.....	.102	.107
2.....	.110	.118
3.....	.123	.137
4.....	.143	.149
5.....	.058	.058
6.....	.070	.070
7.....	.091	.091
8.....	.146	.158
9.....	.085	.090
10.....	.057	.065
12.....	.089	.098
Average (11).....	.098	.104

The results, given in Tables 3 and 4, show clearly that the presence of the carbonate of lime did not affect the accuracy of the nitrate determination, as the differences are within the limit of error.

#### NUMBER OF WASHINGS REQUIRED

Insufficient washing may leave some of the nitric nitrogen in the soil. It was found, with some samples, after seven washings, that subsequent washing removed appreciable amounts of nitrates. Fifteen to twenty washings, with small amounts of water, were found to be sufficient.

#### INTERFERENCE OF CHLORIDES

It is known (2, 5) that the presence of chlorides may affect the accuracy of the determination by the phenol-disulphonic acid method. To study this point, extracts from various cultures were prepared, and sodium chloride added to aliquots. The results were compared with those secured on aliquots which had received no additions of sodium chloride. The results are given in Table 5. Amounts of chlorides up to 100 parts per million did not affect the results within the limit of error, but larger amounts reduced the quantity of nitrates found. Soils of the humid regions are not likely to contain enough salt to affect the results. Some soils of arid sections may contain more than 100 parts per million of chlorides, which may be precipitated by silver sulphate, as recommended by Harper (5) if the phenol-disulphonic acid method is to be used, but it would probably be easier to use one of the zinc ferrous-sulphate methods described on a subsequent page.



Table 5. Nitric nitrogen in parts per million, in cultures, alone and with addition of chlorides

Lab. No.	No addition	50 p.m. Chlorine	100 p.m. Chlorine	200 p.m. Chlorine	500 p.m. Chlorine	1000 p.m. Chlorine
29423 Upland blackland.....	500	500	500			
29423 Upland blackland.....	500		500	490		
29423 Upland blackland.....	460				450	410
28011 Subsoil (Subsoil to 28010).....	80	78	76			
28011 Subsoil (Subsoil to 28010).....	78		74	74		
31886 Duval fine sandy loam, deep phase.....	45	41	39			
31886 Duval fine sandy loam, deep phase.....	84		78	76		
31323 Amarillo silty clay loam.....	152	148	140			
31323 Amarillo silty clay loam.....	145		138	135		
31324 Amarillo silty clay loam.....	31	29	28			
31324 Amarillo silty clay loam.....	29		27	28		
31322 Amarillo silty clay loam.....	105	102.5	102.5			
31322 Amarillo silty clay loam.....	105		100	98		
31326 Amarillo silty clay loam.....	123				103	92
31331 Amarillo fine sandy loam.....	82				64	60
31880 Duval fine sandy loam.....	74				59	50
31888 Webb fine sandy loam.....	60				50	45
22230 Kirkland clay loam.....	156				128	120

### PREFERABLE ALKALI FOR DEVELOPING COLOR

Ammonium hydroxide, sodium hydroxide, or potassium hydroxide can be used for the development of the yellow color in the phenol-disulphonic acid method. These three reagents were tried and ammonium hydroxide was found to be most satisfactory. It produces a clear yellow solution, and is not disagreeable to handle. Ammonia fumes may be evolved, but this difficulty is overcome, to a large extent, by using dilute ammonia. Carbonates sometimes present in the sodium or potassium hydroxide, produce a turbidity in the yellow solution, which renders comparison with the standard difficult. They also make glassware very slippery and hard to handle, which is especially undesirable when a large number of determinations are being made.

### THE PHENOL-DISULPHONIC ACID

Various proportions of phenol and sulphuric acid are used in the preparation of the phenol-disulphonic acid. A mixture of 15 grams of phenol with 100 cc. of sulphuric acid, heated in boiling water for 6 hours, was used in this work. The reagent was satisfactory and not viscous but easily measured. It was found that 2 cc. of the acid is necessary to saturate the residues; 1 cc., as recommended by some workers, is not sufficient for this purpose.

### QUALITY OF PHENOL

A bluish color was occasionally found to occur in some of the preparations of phenol-disulphonic acid. Usually this color disappeared when ammonia was added to develop the yellow color, but with one lot of phenol the color was persistent and so interfered with the yellow color that the phenol had to be discarded. Seven other lots of C. P., or reagent quality phenol obtained from several different manufactur-

ers, were tested against one another in 24 estimations of nitric nitrogen on various samples of soil. Each of these was found to be satisfactory.

### THE POSSIBILITY OF INTERFERENCE BY ORGANIC MATTER

In some instances, it was observed that the yellow color, which was formed upon the neutralization of the phenol-disulphonic acid residue with the ammonium hydroxide, would fade rapidly. The phenol-disulphonic acid method gave decidedly lower results than the Tiemann-Schulze method on aliquots taken from the soil extracts of some of these cultures.

Chamot et al. (2) state that iron in the filtrate would cause trouble. However, a qualitative test of some of the filtrates showed them practically free of iron, which was to be expected, since any soluble iron salts would have been precipitated by the calcium hydroxide used to flocculate the soil particles.

It was thought that the low results were perhaps due to soluble organic substances which might be eliminated by the addition of some oxidizing reagent to the filtrate. To test this theory, additions of potassium permanganate (9), of hydrogen peroxide, and of bromine water were made to aliquots of the filtrates from selected cultures of soils. They were evaporated to dryness and the analysis completed in the usual way. Some results are in Table 6. The addition of potassium permanganate or of hydrogen peroxide increased the amounts of nitric nitrogen. The hydrogen peroxide gave higher results than the permanganate of potash. Bromine gave lower results.

Table 6. Effect of oxidizing reagents on the colorimetric phenol-disulphonic acid method

Treatment	Nitric nitrogen parts per million	Soil number
No addition . . . . .	46	29208
4 drops potas. permanganate . . . . .	92	29208
6 drops hydrogen peroxide . . . . .	126	29208
6 drops bromine water . . . . .	18	29208
No addition . . . . .	2	29209
4 drops potas. permanganate . . . . .	7	29209
6 drops hydrogen peroxide . . . . .	9	29209
6 drops bromine water . . . . .	0	29209
No addition . . . . .	169	29423
4 drops potas. permanganate . . . . .	210	29423
6 drops hydrogen peroxide . . . . .	425	29423
6 drops bromine water . . . . .	128	29423
No addition . . . . .	235	29424
4 drops potas. permanganate . . . . .	235	29424
6 drops hydrogen peroxide . . . . .	450	29424
6 drops bromine water . . . . .	175	29424

Considerable study was given to methods based upon the oxidation of the soil extract with hydrogen peroxide, often with quite satisfactory results, but sometimes the results were not satisfactory. The differences were afterwards found to be partly caused by the presence of

nitrites, which were oxidized to nitrates by hydrogen peroxide, and partly to the production of an interfering color by the hydrogen peroxide. After a large number of tests, the use of hydrogen peroxide was abandoned.

### ADAPTATION OF THE ZINC FERROUS-SULPHATE METHOD TO SOILS

The uncertainty with respect to the accuracy of the phenol-disulphonic acid method rendered it necessary to find another method with which to compare the results. The Tiemann-Schulze method was used at first, but required much time and was not altogether satisfactory. The A. O. A. C. zinc ferrous-sulphate method (1) was tested, and after considerable preliminary work, methods of manipulation were devised which gave satisfactory results. Two procedures were used on the soil extract. If the amount of ammonia expected was large, it was distilled into standard acid and titrated. If the amount of ammonia expected was small, it was estimated by the Nessler method.

**Connecting bulb tubes.** Preliminary blank tests showed the importance of selecting effective Kjeldahl bulb tubes for use in the distillation. Tests showed the ordinary Kjeldahl bulb tube to be unsatisfactory. The ordinary Kjeldahl bulb tubes were then compared with the Clark bulb and the McHargue bulb, with the results given in Table 7. These include the blank on the reagents as well as the effect of the bulb tubes. Use of the ordinary bulb tube may cause erratic results, when the quantity of ammonia distilled is small. Since 1 cc. of the 0.2N acid on 50 grams of soil is equal to 56 parts per million of nitric nitrogen, it is seen from the table that the use of the ordinary bulb may introduce an error of as much as 56 parts per million, while the error with the Clark bulb may be only 11 parts per million. The McHargue bulb gave much better results than the ordinary bulb tube, but the Clark bulb tube gave the lowest blank and the least variation and was adopted for use in our work.

Table 7. Comparison of blanks on Kjeldahl distillation bulb tubes, in cubic centimeters of 0.2 N acid

	Ordinary tube cc.	Clark tube cc.	McHargue tube cc.
Average, 3 tests, Set 1.....	.97	.17	.35
Average, 3 tests, Set 2.....	.63	.22	.39
Average, 3 tests, Set 3.....	.82	.26	.41

**The zinc ferrous-sulphate titration method.** Preliminary work seemed to show that it would be advisable to use hydrogen peroxide to destroy the organic matter in the soil extract used in the zinc ferrous-sulphate method, but further work showed that its use gave erratic and unsatisfactory results. Sodium peroxide, which was recommended by Whiting

et al. (11), was also found to be unsatisfactory, as results were much too low in many cases. The use of both reagents was abandoned, after considerable work, which will not be detailed.

The use of 2 cc. of strong caustic soda in the preliminary distillation to remove the ammonia was insufficient, giving high results in the subsequent reduction. This was probably due to the incomplete decomposition of organic compounds which yield ammonia. The use of 10 to 20 cc. of strong caustic soda (1.43 sp. gr.), followed by distillation to remove the ammonia, then by reduction of the nitrates, and distillation of the ammonia produced from the nitrates, proved quite satisfactory. This method is based upon the Jones method (6) for nitrates in mixed fertilizers. The larger amount of the strong caustic soda not only removes ammonia, but also decomposes organic nitrogenous substances which might interfere with the reduction of nitrates or yield ammonia in the subsequent distillation.

Table 8. The nitrates by the colorimetric method compared with nitrates by the modified zinc ferrous-sulphate titration method when nitrites are absent.  
Nitric nitrogen in parts per million of cultures

Colorimetric method	Zinc ferrous-sulphate method
432	429
179	176
112	115
144	139
250	261
166	163
416	406
163	155
344	361
262	267
280	274
176	174
392	386
67	69
368	346
160	160
456	439
82	82
272	271
44	45
384	392
208	204
408	400
70	68
64	66
480	470
131	133
157	156
368	378
54	50
368	370
54	59
510	492
104	106
344	343
132	143
172	177
80	84
490	492
384	399
230	231
48	50
114	121
140	145

Solutions obtained from cultures of soils which received additions of ammonium sulphate, and which contained large amounts of nitrates, were used in one series of tests. Some of the results of these trials are given in Table 8 and show that there is a close agreement between the two methods, and that the results agree within the limit of error.

**Nesslerizing when the quantity of nitrates was small.** The very small amounts of nitrate in some soils could not be accurately measured with the zinc ferrous-sulphate method of titration, since 1 cc. of 0.2 acid equals 56 parts per million of nitric nitrogen. The Nessler method was adapted to estimating the small amounts of nitric nitrogen which usually occur in field soils, as described elsewhere.

Table 9. Nitric nitrogen in parts per million by the colorimetric method and by the zinc ferrous-sulphate Nessler method, nitrites absent

Colorimetric method	Zinc ferrous-sulphate Nessler method
12.5	13.1
27.5	25.4
1.9	3.0
3.5	6.5
11.3	12.3
4.9	6.7
5.5	6.9
2.0	2.1
7.8	7.8
9.3	8.0
8.0	8.0
3.6	5.1
10.3	13.0
25.5	28.1
20.5	22.6
9.8	12.1
10.5	12.7
17.5	17.6
30.6	30.1
15.0	16.8
20.0	19.0
14.5	16.5
20.0	21.8
18.0	18.8
18.0	19.0
23.0	19.8

The 62.5 gm. of soil mixed with lime was extracted with water and the filtrate made up to 250 cc. One portion was used for nitric nitrogen by the phenol-disulphonic acid method. Another portion of 200 cc. was placed in a Kjeldahl flask with 10 cc. of caustic soda and about 300 cc. water. It was distilled as usual, testing the distillate for ammonia with Nessler's reagents at intervals. After all the ammonia was driven off, the gas was turned off; then, 2.5 grams of zinc dust, 1 gram of ferrous sulphate, and a small piece of paraffin, with ammonia-free-water, were added and the distillation performed as usual. The distillate was collected in 250 cc. volumetric flasks to a little below the mark. It was then made to the mark with ammonia-free water. Of this, 50 cc. was transferred to a 100-cc. flask and diluted with



ammonia-free water; 2 cc. of Nessler's reagent was added and made to the mark. The solution was then compared with the standard in the colorimeter. Some of the results of the comparison with the phenol-disulphonic acid method are in Table 9, showing reasonably close agreement. These estimations were made on cultures of soils. While some of the results do not agree as well as could be desired, most of them agree within the limit of error.

#### ESTIMATION OF NITRATES IN THE PRESENCE OF NITRITES

As already stated, the phenol-disulphonic acid method gave low results with some cultures of soil. This loss at first was thought to be due to the presence of soluble organic matter in the soil solution, but later it was found that nitrites were present, (3) and that the nitrous nitrogen was not estimated by the phenol-disulphonic acid method, while it was included in the results secured by the Tiemann-Schulze or the reduction method.

Table 10. Nitric and nitrous nitrogen by the colorimetric methods compared with the nitrogen by the zinc ferrous-sulphate titration method, in parts per million of soil cultures

Laboratory Number of soils used for cultures	Colorimetric Nitric N.	Colorimetric Nitrous N.	Colorimetric Nitric and Nitrous	Zinc-ferrous sulphate method
5958.....	368	56	424	407
9225.....	66	20	86	98
7346.....	18	22	41	39
7350.....	140	13	153	159
7350.....	98	18	116	115
23361.....	53	141	194	190
23549.....	128	39	167	157
5935.....	66	16	82	95
5937.....	45	22	67	67
5958.....	48	200	248	232
5935.....	106	54	160	154
5958.....	344	14	358	347
20725.....	160	75	235	216
5958.....	147	148	295	268
20720.....	109	3	113	109
20721.....	60	7	67	68
33135.....	65	26	91	87
9277.....	229	23	252	249
33134.....	219	0	219	226
22230.....	60	78	138	141
23321.....	155	82	237	233
23322.....	70	111	181	178
25785.....	219	30	249	253

The alpha-naphthylamine colorimetric method for nitrites was modified to adapt it to soils and is described in full on another page.

The methods were compared on extracts of cultures of soils containing nitrites to which sulphate of ammonia had been added before incubation. Of the 250 cc. soil extract, 10 cc. was taken for nitrite determination with the alpha-naphthylamine method, 10 cc. for nitrate determination with the phenol-disulphonic acid method, and 200 cc. for total nitric and nitrous nitrogen with the zinc ferrous-sulphate titration method. Some of the results are given in Table 10. The agreement indicates the accuracy of the methods. As pointed out in another

part of this paper, the average error in reading the color in the colorimetric method for nitrates in solution is about 5 per cent, while it may run to 13 per cent.

### DETAILED DESCRIPTION OF METHODS

On account of the importance of apparently small details, which may nevertheless save time and increase accuracy, it is considered desirable to give in detail the methods as they were finally used.

#### The Phenol-Disulphonic Acid Method

**Preparation of the standard nitrate solution.** Dissolve 7.25 grams of C. P. potassium nitrate in water, and make up to one liter. Determine nitrogen in 25 cc. with the zinc ferrous-sulphate method. Adjust so that the solution (A) contains 1.0 milligram of nitric nitrogen per one cubic centimeter.

Dilute 10 cc. of A to one liter. This solution (B) contains .01 milligram of nitrogen per cubic centimeter.

Evaporate 5 cc. of solution B to dryness, and saturate the residue with 2 cc. phenol-disulphonic acid. Develop the color with ammonia and make up to volume in a 100-cc. volumetric flask. This standard yellow solution contains .0005 milligram of nitrogen per cubic centimeter.

#### Estimation of Nitric Nitrogen in Soils and Cultures of Soils

To 20 grams of cultures of soils, or 40 grams of field soil or the equivalent if wet, in a beaker, add 40 cc. of distilled water, and about 2 grams of finely ground calcium oxide and stir thoroughly. Allow the soil to settle for 10 to 15 minutes. Filter the supernatant liquid into a 200-cc. volumetric flask provided with a wire between the neck of the flask and the funnel, to allow the displaced air to escape from the flask. Transfer the soil to the filter with as little water as possible, and wash about 15 to 20 times with small portions of water. Make up to the mark and mix thoroughly by placing the thumb on the mouth of the flask and inverting it several times. Rinse a 10-cc. pipette thoroughly twice with the filtrate; then transfer 10 cc. to an evaporating dish and evaporate to dryness on a steam bath. Use 10 cc. of filtrate obtained from cultures of soils or 25 cc. obtained from field soils.

Add 2 cc. of phenol-disulphonic acid to the cool dry residue. Rotate the acid about the dish several times in order to secure thorough contact with all the residue. After 10 minutes, wash the sides of the dish with a fine stream of water and allow the acid solution to cool for about 30 minutes. Transfer the solution carefully to a 100-cc. volumetric flask, add 25 cc. of dilute ammonium hydroxide (1 part of the concentrated ammonia to 4 parts of distilled water), and mix thoroughly. Make to volume with water and again mix thoroughly.

Rinse the comparison tube of the colorimeter twice with small portions of this yellow solution. Match a third portion against freshly prepared yellow standard solution in the colorimeter.

If the color of the solution to be tested is too strong for comparison, take 10, 20, or 25 cc. with a pipette and make up to 50 cc., 100 cc., or 200 cc. in a graduated flask. Then make the comparison as usual.

### The Zinc Ferrous-Sulphate Titration Method

To 50 grams of dry soil, or its equivalent if wet, in a beaker, add 50-60 cc. of distilled water and 2 to 3 grams of finely ground calcium oxide and stir thoroughly. Allow to settle for 10 minutes and then transfer the supernatant liquid first and then the soil to a filter. Wash the soil 20 to 25 times, collecting the filtrate in a Kjeldahl flask. Add 20 cc. of caustic soda (about 1.4 sp. gr.) and enough distilled water to make the volume of the filtrate about 500 cc., and connect to the condenser. Distill off 150 to 200 cc. and then begin to test distillate for ammonia with Nessler's reagent. When no more ammonia can be detected, disconnect and allow to cool.

Then add a small piece of paraffin to prevent foaming, 5 grams of zinc dust, and 2 grams of ferrous sulphate. Connect to the condenser and distill into 12 cc. of .2 N acid. Titrate with .1 N sodium hydroxide; 1 cc. of 0.2 acid equals 56 parts per million of nitric nitrogen, on 50 grams of soil.

Run 2 blanks with each set and subtract the average amount of nitrogen found in the blanks.

Nitric and nitrous nitrogen combined are determined by this method. If nitrites are present, they should be determined separately and the nitrite nitrogen subtracted from the total nitrogen.

### Ferrous Sulphate-Nessler Method

**Preparation of ammonia-free water.** Prepare Nessler's reagent as described below (about 250 cc.) with distilled water if ammonia-free water is not available, and allow to settle over night. In the meantime, prepare distilling apparatus with glass condenser and see that the inner tube is not in direct contact with any rubber tubing. The distilling apparatus should be set up in a room free from ammonia.

Place in a 6-liter flask 10-15 grams of sodium carbonate, a few pieces of ignited pumice stone, and fill it with condensed water. Connect with the distilling apparatus and distill off about one-fourth of the water in the flask. Then collect distillate in 50 cc. portions at a time and test it with 2 cc. of Nessler's reagent. When no more ammonia can be detected, begin to collect the distillate in a flask protected with a U tube, and save as ammonia-free water.

**Preparation of Nessler's reagent:** Dissolve 61.75 grams of potassium iodide in 250 cc. of ammonia-free water, and add a cold solution of

mercuric chloride which has been saturated by boiling with an excess of the salt. About 25 grams of mercuric chloride dissolved in 425 cc. of ammonium-free water will be sufficient for this purpose. Pour the mercury solution cautiously into the potassium iodide solution until a precipitate begins to form. Then with a pipette, drop by drop, add enough solution to make the color a permanent bright red. Dissolve the red precipitate by adding exactly .75 gram of potassium iodide. Stir well, and transfer to a one-liter flask. Then add 150 grams of potassium hydroxide dissolved in 250 cc. of ammonia-free water. Shake well, and make up to the mark with ammonia-free water. Mix thoroughly, transfer to a clean flask, and allow to settle over night. Pour off the supernatant liquid and keep it in a place free from ammonia.

**Preparation of standard ammonium chloride solution:** To 7.15 cc. of .1 N ammonium hydroxide solution, add 3.58 cc. of .2 N hydrochloric acid. Transfer to a liter flask and make up to the mark with ammonia-free water. Mix thoroughly and then transfer to a bottle. 1 cc. of this solution = 0.00001 gram of nitrogen.

**Operation:** Weigh out 50 grams of soil (or 25 grams of soil obtained from cultures containing larger amounts of nitrates) into a beaker. Add about 50 cc. of distilled water, 2 to 3 grams of finely ground calcium oxide; stir, and after 10 minutes, transfer the supernatant liquid and soil to a filter, collecting the filtrate in a Kjeldahl flask, and wash thoroughly about 20 times. Add 10 cc. of caustic soda, enough distilled water to make the volume about 500 cc., and connect to the condenser. Distill off about 200 cc. and then begin to test the distillate with Nessler's reagent. Continue to test until no more ammonia can be detected. Then turn off the heat and put a 250-cc. volumetric flask at the delivery tube to collect the distillate. Disconnect the Kjeldahl flask; add a small piece of paraffin to prevent foaming, 2.5 grams of zinc dust, and 1 gram of ferrous sulphate. Add also a little ammonia-free water if necessary. Connect with the condenser and distill off nearly 250 cc.

Make distillate up to 250 cc. with ammonia-free water. To 50 cc. in a 100-cc. volumetric flask, add about 20 to 30 cc. of ammonia-free water, shake and then add 2 cc. of Nessler's reagent. Make up to the mark, shake, and then allow to stand 10 minutes. Compare a portion of this with the standard in the colorimeter.

For the preparation of the standard solution, dilute 10 cc. of the standard ammonium chloride in a 100-cc. volumetric flask, with 60 to 70 cc. of ammonia-free water, add 2 cc. of Nessler's reagent, and make up to the mark. Shake thoroughly and allow to stand 10 minutes. Then use for comparison in the colorimeter. If Nessler's reagent is added directly to the standard ammonia solution before dilution, a precipitate is formed, which renders the solution useless.

**NITRITE NITROGEN IN SOILS**

**Sulphanilic acid.** Dissolve 1.6 grams of sulphanilic acid in 200 cc. of dilute acetic acid (29 per cent or 1.04 specific gravity). Heat gently and stir thoroughly in order to dissolve the sulphanilic acid. Cool and preserve in a glass-stoppered bottle.

**Naphthylamine acetate.** Dissolve .5 gram of alpha-naphthylamine in 100 cc. of dilute acetic acid (29 per cent or 1.04 specific gravity). Heat gently and stir thoroughly to dissolve the naphthylamine. Cool and place in a brown glass-stoppered bottle. This reagent is good for 2 to 3 days. After this period of time, a new supply should be prepared.

**Silver nitrite.** Dissolve 10 grams of silver nitrate in 20 cc. of hot distilled water (8). Dissolve 10 grams of sodium nitrite in 15 cc. of distilled water and heat. Mix both solutions while hot and stir thoroughly, then allow to cool. Filter and wash about 10 times with ice-cold water. Place the washed precipitate with the filter paper between white blotting paper and press it gently to get the moisture out. Then wrap it well with another filter paper, and dry it in a desiccator in a dark place for a week or longer.

**Standard sodium nitrite solution:** Solution A. Dissolve 0.55 gram of silver nitrite in 50 cc. of distilled water in a beaker by heating. In another beaker dissolve 0.3 gram of sodium chloride in 25 cc. of water. Add the sodium chloride solution to the silver nitrite solution. Stir thoroughly. Allow to cool and then filter into a 500-cc. volumetric flask. Wash thoroughly about 15 times. Then add 1 cc. of chloroform, make it up to the mark, and shake thoroughly. Transfer the solution to a brown bottle and stopper well. Keep in a dark place. Determine total nitrogen in 50 cc. by the zinc ferrous-sulphate method, and nitric nitrogen in 10 cc. by the phenol-disulphonic acid method, and adjust if necessary. One cc. should contain 0.1 mg. of nitrous nitrogen.

Solution B. Dilute 10 cc. of solution A to one liter. This solution contains .001 mg. of nitrogen in 1 cc. Keep in a brown bottle.

**Standard colored solution.** Transfer 10 cc. of solution B to a 100-cc. flask, add about 75 cc. of water and 2 cc. each of the sulphanilic acid reagent and the alpha-naphthylamine acetate. Shake, make up to the mark, and shake again thoroughly. Set aside for 15 or 20 minutes and then compare with the unknown in the colorimeter. One cc. of this pink solution (C) contains 0.0001 mg. of nitrous nitrogen.

**Qualitative Test for Nitrites in Soils**

Prepare soil solution as described under the colorimetric nitrate method, using 20 grams of dry soil (or equivalent of a wet soil), and 2 to 3 grams of calcium oxide. Dilute after filtration to 200 cc. in a



graduated flask. Transfer 2 cc. of this solution to a test tube marked at 10 cc. Make up slightly below the mark with distilled water. Add 5 drops each of the sulphanic acid and of the alpha-naphthylamine acetate. Shake thoroughly. Allow color to form 15 to 20 minutes. Then compare with 10 cc. of the standard (C) in another test tube. The standard is equal to 5 parts per million of nitrous nitrogen, in the soil taken.

### Quantitative Determination of Nitrous Nitrogen

First observe the unknown in the qualitative test. If a heavy precipitate has been formed, make up 10 cc. of the soil solution to 200 cc. Transfer 10 cc. of this dilution to a 100-cc. volumetric flask, dilute to 75 cc. and add 2 cc. of each reagent. Make it to the mark and shake it as usual. Allow color to form 15 to 20 minutes. Then compare with the standard in the colorimeter. If the color of the unknown is still too deep, dilute 25 cc. of this to 50 cc. or to 100 cc. and compare as usual.

If only a slight precipitate is formed in the qualitative test, make up 10 cc. of the soil extract to 100 cc., transfer 10 cc. to a 100-cc. flask, and develop the color as previously described.

If no precipitate is present in the qualitative test, transfer 10 cc. to a 100-cc. volumetric flask, and dilute and develop the color. If the color is too deep, make up 10 cc. of the soil solution to 100 cc.; place 25 cc. of this dilution to a 100-cc. flask, and develop the color.

The pink color should always be developed in a 100-cc. volumetric flask.

In case of field soils, or soils low in nitrites (lower than 5 parts per million), determine the nitrites directly in the filtrate from the soil, without dilution.

### SUMMARY

Differences in the quantities of nitrogen found by the phenol-sulphonic acid method and by the Tiemann-Schulze method, and by reduction methods were found to be chiefly due to the presence of nitrites, in large quantities, in some cultures, which were not included in the results by the phenol-disulphonic acid method, but were included with the other methods.

For the purpose of checking the colorimetric method, the zinc ferrous-sulphate method for combined nitrate and nitrite nitrogen was adapted for use with soils. When moderate amounts of nitrogen were present, the ammonia was collected in acid and the acid titrated with alkali. For small amounts of ammonia, the ammonia in the distillate was estimated by the Nessler method. Both methods are described in detail.

Details of the alpha-naphthylamine method for nitrites was modified to adapt it to estimation of nitrites in soils.

When nitrites were allowed for, the results by the phenol-disulphonic

acid method checked very well against the zinc ferrous-sulphate methods.

The Clark bulb was superior to the ordinary Kjeldahl distillation bulb.

The error due to matching the color with the standard in the phenol-disulphonic acid method for nitric nitrogen was found to average about 5 per cent of the amount of nitrogen present, while it was sometimes found to be as much as 13 per cent.

Lime was a good flocculating agent for soils in nitrate work. Potash alum may be used, but was less satisfactory.

The presence of calcium carbonate did not appear to affect the results by the colorimetric phenol-disulphonic acid method.

Washing the soil residue 15 to 20 times is recommended.

Ammonium hydroxide for developing the yellow color is preferable to sodium or potassium hydroxides.

Additions of oxidizing agents such as hydrogen peroxide gave higher results for nitrates on some of the cultures, when the colorimetric method was used, but their use was found to be unsatisfactory.

Ordinarily the colorimetric methods for nitrates and for nitrites should be used for soils or cultures secured in nitrification experiments. The zinc ferrous-sulphate methods may be used for testing the method or for special purposes.

On account of the importance of apparently minor points to secure accurate results or to save the time of the analyst, the methods for nitrates and nitrites as finally used are described in detail.

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