DETERMINATION OF NITROGEN IN SOILS.
PART V. ESTIMATION OF TOTAL NITROGEN TO INCLUDE NITRATES.

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In the previous communications (Sreenivasan, *Ind. J. Agric. Sci.*, 1932, **2**, 525; *ibid.*, 1934, **4**, 320, 546; Sreenivasan and Subrahmanyan, *Ind. J. Agric. Sci.*, 1933, **3**, 646) it has been shown that accurate estimates of organic nitrogen can be obtained and the time of digestion greatly reduced by pre-treating soil with dilute sulphuric acid combined with small quantities of oxidising agents such as peroxides, potassium permanganate or dichromate. The values thus obtained will not, however, include the minute quantities of nitrates that are present in most soils. The present enquiry was undertaken, therefore, with the object of combining the reduction of nitrate by some pre-treatment with oxidation during digestion.

Several methods have been proposed for the estimation of nitrates in soils and some of them have been specially designed for inclusion with the Kjeldahl method of acid digestion. All the methods have the common feature that they involve the reduction of nitrate to ammonia. The reagents generally employed for that purpose are (*a*) ferrous sulphate; (*b*) salicylic acid together with zinc dust or sodium thiosulphate; (*c*) zinc, Devarda's alloy, zinc-copper couple or reduced iron in acid media; and (*d*) aluminium or Devarda's alloy in alkaline media. Among the various modifications, particular attention may be drawn to the salicylic acid methods of Jodlbauer (*Chemiches Central-blatt*, 1886, **3**, 433), Förster (*Landw. Versuchs-Stat.*, 1891, **38**, 165) and Ranker (*Ann. Mo. Bot. Gardens*, 1926, **12**, 367), the acid reduction methods of Ulsch (*Z. anal. Chemie*, 1891, **30**, 175) and Olsen (*Compt. Rend. Trav. Lab. Carlsberg*, 1927, **17**, No. 3) and the alkali reduction methods of Burgess (*Univ. Calif. Publications, Agric. Scr.*, 1914, **1**, 51), Allen (*Ind. Eng. Chem.*, 1915, **7**, 521), Davisson and Parsons (*Ind. Eng. Chem.*, 1919, **11**, 306) and Payne (*J. Agric. Sci.*, 1927, **17**, 153). While these methods are generally satisfactory in presence of small amounts of nitrate, most of them are not applicable when the quantities of nitrates are comparatively large. Thus, Mitscherlich and Herz (*Landw. Jahrb.*, 1909, **38**, 279, 533), Gallagher (*J. Agric. Sci.*, 1923, **13**, 63), Pucher, Leavenworth and Vickery (*Ind. Eng. Chem., Anal. Edn.*, 1930, **2**, 191) and others, have drawn attention to the inadequacy of the salicylic acid method, while the Ulsch method of acid reduction (*loc. cit.*) has been criticised by Buhlert and Fickendey (*Landw. Versuchs-Stat.*, 1909, **63**, 239), Burgess (*loc. cit.*),

Davisson and Parsons (*loc. cit.*) and others. Burgess proposed a method of reduction with aluminium in faintly alkaline medium, but Allen (*loc. cit.*) found it to be unsatisfactory. Later, Van Wijk (*Soil Sci.*, 1924, **17**, 163) claimed to have applied that method with some success.

In view of the above and the need for further information regarding the relative merits of different reducing agents, it was considered necessary to conduct some preliminary trials with soil suspensions containing known quantities of nitrate.

EXPERIMENTAL.

Comparative efficiencies of some of the reagents commonly used for reduction in acid media.—A specimen of black cotton (surface) soil from Indore was air-dried and ground to pass the 30-mesh sieve. Samples (5 g.) of the soil were treated with (*a*) ferrous sulphate (1 g.); (*b*) zinc powder (0.5 g.); (*c*) Devarda's alloy (0.5 g.); and (*d*) salicylic acid (1 g.) followed by zinc powder (1 g.) respectively and the digestions conducted by 'dry' and 'wet' methods in the manner outlined in a previous communication (Sreenivasan, *loc. cit.*, 1932). The results thus obtained have been presented in Table I.

TABLE I.

Mode of digestion	Nitrogen in parts per million (Averages)				
	Soil untreated (Control)	Reduction with			
		Ferrous sulphate	Zinc powder	Devarda's alloy	Salicylic acid and zinc
'Dry' (Official) ..	388·2	395·0	395·0	395·0	406·1
'Wet' (Overnight) ..	532·5	532·5	534·7	532·5	523·6

It may be seen from the above that treatment with reducing agents combined with 'dry' digestion leads to increased estimates of total nitrogen. In the case of 'wet' digestion, however, there is comparatively little difference between the different values so that it is difficult to say whether any of the nitrate is included in the estimates obtained by that method.

Digestion in presence of various reducing agents to include small quantities of nitrate.—Since most soils contain only traces of nitrate, it was considered that the most satisfactory method of testing the different treatments would be to work with soils containing known amounts of nitrates.

TABLE II.

Reduction with	Mode of digestion	Cuttack, Lowland (Paddy), surface soil				Sholapur, light clay, surface soil				Indore, heavy black, surface soil			
		Soil alone (control)	Soil+40 p.p.m. of N as nitrate	Soil+80 p.p.m. of N as nitrate	Soil+100 p.p.m. of N as nitrate	Soil alone (control)	Soil+40 p.p.m. of N as nitrate	Soil+80 p.p.m. of N as nitrate	Soil+100 p.p.m. of N as nitrate	Soil alone (control)	Soil+40 p.p.m. of N as nitrate	Soil+80 p.p.m. of N as nitrate	Soil+100 p.p.m. of N as nitrate
Ferrous sulphate (5g.)	'Dry' (Official)	758·8	796·4 (37·6)	827·4 (68·6)	840·7 (81·9)	301·7	337·3 (35·6)	377·3 (75·6)	392·7 (91·0)	395·0	432·6 (37·6)	481·4 (86·4)
	'Wet' (Modified)	767·6	803·0 (35·4)	829·6 (62·0)	840·7 (73·1)	337·2	374·9 (37·7)	403·8 (66·6)	410·4 (73·2)	532·5	570·2 (37·7)	598·9 (66·4)
Zinc powder (2g.)	'Dry' (Official)	756·6	796·4 (39·8)	834·1 (77·5)	849·7 (93·1)	..	..	..	..	..	..	..
	'Wet' (Modified)	767·6	805·2 (37·6)	838·5 (70·9)	851·9 (84·3)	..	..	..	..	..	..	..
Devarda's alloy (2g.)	'Dry' (Official)	754·3	794·1 (39·8)	829·6 (75·3)	847·4 (93·1)	306·1	339·5 (33·4)	379·4 (73·3)	395·0 (89·1)	395·0	430·3 (35·3)	485·8 (90·8)
	'Wet' (Modified)	767·6	803·0 (35·4)	842·9 (75·3)	851·9 (84·3)	337·2	377·1 (39·9)	412·6 (75·4)	425·9 (88·7)	532·5	572·4 (39·9)	619·0 (86·5)

The figures within brackets refer to the amounts (as p.p.m.) of nitrate-nitrogen reduced by the respective reagents.

5 g. lots of three different soils used in a previous study (Sreenivasan, *loc. cit.,* 1934) were treated with potassium nitrate solution in quantities corresponding to 40, 80 and 100 parts per million of nitrogen (on the weight of the soil) and the digestions carried out by 'dry' and 'wet' methods after reduction with different reagents. In all the cases, the reduction was allowed to proceed overnight before the digestion was commenced. The samples to be 'dry' digested were evaporated to dryness on the water-bath before adding concentrated sulphuric acid. The reducing agents employed, as also the values obtained for total nitrogen, are given in Table II.

It may be noted that in all the cases fairly accurate estimates were obtained when the quantities of nitrate were small. When the nitrate content of the soil was increased, the reduction did not proceed to completion. This was particularly so in the cases of specimens treated with ferrous sulphate. Reduction with zinc dust was carried out only in the case of one soil, as it was found that there was no appreciable difference between the results obtained with that reagent and those with Devarda's alloy.

Another observation of interest is that reduction of nitrate proceeds more efficiently under conditions of 'dry' digestion than under those of the 'wet' treatment. On the other hand, digestion of organic nitrogen is greatly facilitated by the 'wet' treatment so that the estimates of total nitrogen obtained by that method are invariably higher than those obtained by 'dry' digestion.

Estimation of total nitrogen in presence of larger quantities of nitrate.—In the following experiments, the black cotton soil (5 g.) from Indore was treated with quantities of nitrate corresponding to 160, 200, 300 and 400 parts per million of nitrogen respectively, and the total nitrogen estimated by 'dry' and 'wet' methods, using ferrous sulphate or Devarda's alloy for reduction. The results have been presented in Table III.

It would be seen from the above that ferrous sulphate is quite unsatisfactory especially under conditions of 'wet' digestion. Devarda's alloy is also comparatively inefficient when used in acid media.

Effect of addition of Devarda's alloy at fume-stage.—Since it was observed that reduction of nitrates was generally more complete with the 'dry' digested specimens than with the 'wet' treated ones, it was thought desirable in the latter cases to add the reducing agent after all the water was driven out. Specimens (5 g.) of the Indore soil were treated with varying amounts of nitrate and the mixtures heated with water and sulphuric acid as in the usual 'wet' digestion. At the time water acid fumes just began to evolve, the Kjeldahl flasks were cooled and 3–4 g. of Devarda's alloy added to each. The contents of the

TABLE III.

Reducing agent used	Mode of digestion	Nitrogen in parts per million (Averages)				
		Soil alone (Control)	Soil + 160 p.p.m. of N as nitrate	Soil + 200 p.p.m. of N as nitrate	Soil + 300 p.p m. of N as nitrate	Soil + 400 p.p.m. of N as nitrate
Ferrous sulphate (5 g.)	'Dry'	395·0	508·0 (113·0)	534·6 (139·6)	561·3 (166·3)	....
	'Wet'	532·5	....	541·3 (8·8)	541·3 (8·8)	....
Devarda's alloy (3-4 g.)	'Dry'	395·0	532·5 (137·5)	565·7 (160·7)	661·1 (266·1)	749·9 (354·9)
	'Wet'	532·5	685·4 (152·9)	703·2 (170·7)	794·2 (261·7)	856·3 (323·8)

The bracketted figures represent the quantities of nitrate nitrogen (in p.p.m.) included in the estimate.

flasks were then heated to complete digestion. It was observed that when the quantities of added nitrate corresponded to 200, 300 and 400 p.p.m. of nitrogen, the amounts reduced in presence of the soil by the above procedure were 135.3, 199.6 and 283.9 p.p.m. respectively, thereby showing that nitrate reduction was even less complete than in the previous experiments. The very low values are no doubt due to the partial volatilisation of nitric acid even before addition of the reducing agent.

It may thus be seen that, in acid media, although reducing agents were fairly effective in presence of small quantities of nitrate, they failed badly when the quantities were comparatively large. This was rather surprising, especially in view of the fact that, in the absence of the soil, nitrate solutions containing up to 400 p.p.m. of nitrogen were almost quantitatively (97–100 per cent.) reduced by those reagents.

Effect of refluxing soil-nitrate mixture during reduction in acid media.—It first appeared probable that in the previous experiments some of the nitric acid, which is the first product, may have been lost by volatilisation owing to the large surface presented by the soil. With a view to avoiding such a loss, the reduction was carried out in Kjeldahl flasks fitted with reflux condensers. In one set of experiments, the refluxing was done for 30–45 mins. after addition of the reducing agent while, in another, the latter was added in small quantities at a time. In either case, when reduction was over, the 'wet' digestion was completed as usual. The results are given in Table IV.

TABLE IV.

Mode of reduction	Nitrogen in parts per million (Averages)					
	Soil-lowland (paddy), surface, Cuttack			Soil-heavy black, surface, Indore		
	Soil alone (Control)	Soil + 200 p.p.m. of N as nitrate	Soil + 400 p.p.m. of N as nitrate	Soil alone (Control)	Soil + 200 p.p.m. of N as nitrate	Soil + 400 p.p.m. of N as nitrate
Devarda's alloy (5 g.) without refluxing	767·6	936·3 (168·7)	1082·0 (314·4)	532·5	703·2 (170·7)	856·3 (323·8)
Devarda's alloy (5 g.) with refluxing	767·6	932·4 (164·8)	1096·0 (328·4)	532·5	....	....
Devarda's alloy (5 g.) added in instalments with refluxing	767·6	940·6 (173·0)	1109·0 (341·4)	532·5	718·8 (186·3)	887·4 (354·9)

The bracketted figures represent p.p.m. of nitrate nitrogen reduced in each case.

It may be noted that although refluxing combined with the addition of the reducing agent in small instalments led to improved estimates, the reduction was still incomplete.

Loss of nitrogen during acid reduction of nitrate in presence of soil.—With a view to finding whether in the course of the above experiments, some of the nitrate nitrogen was lost in the gaseous state (either as nitric oxide or as nitrogen peroxide), some experiments were conducted with soils treated as above and the escaping gases tested for (1) nitric oxide, by absorbing in standard permanganate solution and titrating the latter after absorption, and (2) nitrogen peroxide, by absorbing in dilute alkali and testing for nitrate and nitrite in the latter. Negative tests were obtained in both the cases.

Ulsch (*loc. cit.*) drew attention to the fact that in presence of zinc and sulphuric acid, nitrates are first reduced to nitrites, which lead to some loss of nitrogen. Gallagher (*loc. cit.*) suggested that acid reduction of nitrates in presence of amino-bodies may lead to loss of nitrogen in elementary form by interaction of the latter with the nitrous acid formed in the intermediary stages of nitrate reduction. It appeared probable that similar reactions might have occurred in presence of the soil, the humic nitrogen providing the free amino groups that would react with the nitrous acid.

With a view to finding the extent to which the nitrogen may be lost in presence of amides or amino-bodies, some experiments were carried

out digesting mixtures containing known quantities of nitrate with given amounts of (1) acetamide; (2) glycine; (3) asparagine; and (4) casein, after preliminary reduction with Devarda's alloy and acid (Table V).

TABLE V.

Nitrate nitrogen added (mg.)	Total Nitrogen (in mg.) (Averages)							
	Acetamide		Glycine		Asparagine		Casein	
	Expected	Found	Expected	Found	Expected	Found	Expected	Found
1·00	2·07	1·94	3·19	3·09	4·04	3·93	4·34	4·19
2·00	3·07	2·79	4·19	3·96	5·04	4·79	5·34	5·08
5·00	6·07	5·43	7·19	6·53	8·04	7·60	8·34	7·92

It would be seen from the above that in all the cases there is loss of nitrogen similar to what occurs in presence of the soil.

Reduction of nitrate in presence of dilute acid.—In the foregoing experiments, nitrate reduction was done in presence of 1 : 1 sulphuric acid. Since a more dilute acid would be better ionised and consequently more reactive, it was thought that the efficiency of the reduction could be improved by using either 1 : 10 or 1 : 30 acid. Some experiments were accordingly carried out treating solutions containing known quantities of nitrate with excess of sulphuric acid of the above strengths and 4–5 g. of Devarda's alloy in each case (Table VI).

TABLE VI.

Nitrate (as mg. of N)	0·51	1·02	1·53	2·04
Per cent. reduction with 1 : 30 H_2SO_4 ..	98·7	98·7	96·4	97·7
Per cent. reduction with 1 : 10 H_2SO_4 ..	100·0	98·7	99·7	99·6

The results show that the reduction is very nearly complete in presence of 1 : 10 acid. The more dilute acid (1 : 30) is not so useful.

To verify whether loss of nitrogen of the type mentioned above will occur even when dilute (1 : 10) acid is used for reduction, a few trials were carried out with mixtures of solutions of asparagine and nitrate. After reduction of nitrate, the digestions were conducted in the usual way (Table VII).

TABLE VII.

Nitrate (as mg. of N) added	..	..	0·0	1·02	2·04
Nitrogen estimated (in mg.)	..	..	3·19	4·03	4·97
Nitrogen expected (in mg.)	..	..	....	4·21	5·23

It may be seen that low values are obtained even when very dilute acid is used for reduction so that it would appear that for estimation of total nitrogen including nitrate-nitrogen in soils, any method of reduction involving intermediary formation of nitrous acid is unsatisfactory and will invariably yield low values.

The salicylic acid method.—Since this method involves only the formation of the non-volatile nitro-salicylic acid and its subsequent reduction (by zinc or thiosulphate) to the corresponding amino acid, it was thought that it would be free from the defects observed in the earlier experiments. Some trials were carried out, therefore, using samples (5 g.) of the Indore soil treated with varying amounts of nitrate. The treatment with salicylic acid as also the subsequent reduction with zinc dust (*A. O. A. C.*, 1930) were carried out under 'dry' as well as 'wet' conditions. The digestions were then completed in the usual way (Table VIII).

TABLE VIII.

Mode of reduction and digestion	Nitrogen in parts per million (Averages)				
	Soil alone (Control)	Soil + 80 p.p.m. of N as nitrate	Soil + 100 p.p.m. of N as nitrate	Soil + 200 p.p.m. of N as nitrate	Soil + 400 p.p.m. of N as nitrate
Salicylic acid (1 g.) + zinc dust (1 g.) 'Dry' digested	406·1	472·5 (66·4)	496·9 (90·8)	581·3 (175·2)	789·7 (383·6)
Salicylic acid (1 g.) + zinc dust (1 g.) 'Wet' digested	523·6	588·0 (64·6)	612·9 (89·3)	....	841·0 (317·4)

The figures in brackets represent nitrate nitrogen (in p.p.m.) reduced in each case.

The salicylic acid method yielded not only low but also highly discordant values especially after 'wet' digestion. It appeared probable that the nitric acid which was first formed, was too dilute to effectively nitrate the salicylic acid so that it was subsequently lost by volatilisation. This conclusion was supported by the observation that nitrous

fumes (HNO_3 and NO_2) could be detected in the escaping vapours. Moreover, separate experiments carried out with salicylic and nitric acids of different strengths showed that nitration of the former was never complete in presence of water.

The foregoing observations are in accordance with those of some of the previous workers (Mitscherlich and Herz, *loc. cit.;* Gallagher, *loc. cit.;* Pucher *et al, loc. cit.;* and others). It has been suggested by Ranker (*J. Assoc. Off. Agric. Chemists,* 1927, **10**, 230) that accurate estimates can be obtained if the specimens are first evaporated to complete dryness on the water-bath under vacuum. Such a procedure is inconvenient and will be difficult to follow especially when there are large numbers of determinations to be carried out.

Reduced iron method.—Schenke (*Chem. Ztg.,* 1893, **17**, 977) and Greaves and Hirst (*Soil Sci.,* 1917, **4**, 179) recommended the Ulsch-Kjeldahl method (*loc. cit.*) for determination of total nitrogen. Oslen (*loc. cit.*) reported that nitrate nitrogen even up to 1,000 p.p.m. was completely reduced by *ferrum reductum* in presence of sulphuric acid. With a view to verifying this, 5 g. lots of the three soils used in previous studies were treated with varying amounts of nitrate and the reduction carried out according to Olsen using 5 g. of pure reduced iron in each case. The suspensions were then digested in the usual way. The results are given in Table IX where allowance has been made for the nitrogen (0.004 per cent.) present in the specimen of reduced iron.

TABLE IX.

Soil used	Nitrogen in p.p.m. (Averages)		
	Soil alone (Control)	Soil + 203·2 p.p.m. of N as nitrate	Soil + 406·4 p.p.m. of N as nitrate
Lowland, surface—Cuttack	764·7	962·7 (198·0)	1149·0 (384·3)
Heavy black, surface—Indore	534·0	725·5 (191·5)	923·4 (389·4)
Light clay, surface—Sholapur	336·2	530·1 (193·9)	717·6 (381·4)

The figures in brackets refer to nitrate (as p.p.m. of N) reduced in each case.

It would be seen that fairly accurate values are obtained by the method, more than 95 per cent. of the added nitrate being included in the estimate of total nitrogen. The method was, however, tedious in view of the fact that a very large amount of iron was found necessary

and offered difficulties during distillation on account of the bulky ferric hydroxide being precipitated. Moreover, since reduced iron itself contains some nitrogen, a blank determination had always to be carried out.

Reduction in alkaline media.—Some previous workers (Devarda, *Z. anal. Chem.*, 1894, **33**, 113; Mitscherlich and Herz, *loc. cit.*; Cahen, *Analyst*, 1910, **35**, 307; Van Slyke, *J. Biol. Chem.*, 1911, **10**, 15; Valmari, *Bied. Zentr.*, 1914, **43**, 217; Burgess, *loc. cit.*; Knecht, *J. Soc. Chem. Ind.*, 1915, **34**, 126; Allen, *loc. cit.*; Gallagher, *loc. cit.*; Van Wijk, *loc. cit.*; Payne, *loc. cit.*; and others) have drawn attention to the fact that reduction of nitrate proceeds more effectively in alkaline than in acid media. With a view to determining whether any one of them was particularly suitable for the estimation of organic nitrogen, some experiments were first carried out using Devarda's alloy in alkaline medium for reduction. It was observed that when known amounts of nitrate solution (with and without soil) were treated with even minute quantities of Devarda's alloy in presence of 3–4 per cent. alkali, the reduction proceeded rapidly in the cold and was complete in 1½ to 2 hours. Use of stronger alkali resulted in considerable volatilisation of ammonia, while, with a more dilute alkali, the reduction proceeded very slowly. Even with 4 per cent. alkali, it was found essential to safeguard against volatilisation of traces of ammonia by the use of some acid trap. This was achieved by using a rubber stopper carrying a bent thistle funnel with a little glass-wool soaked in dilute (1 : 10) sulphuric acid. After reduction was complete, the acid in the funnel was washed into the Kjeldahl flask with small quantities of water. Concentrated sulphuric acid (20 c.c.) was then added to the soil suspension and the digestion completed as usual. It was found that although the method worked out satisfactorily, there was always a danger of back-suction of acid if there was any fall of pressure in the Kjeldahl flask before the reduction was complete. Moreover, the use of a separate trap was often inconvenient. Consequently a simpler device was tried in which one or two circular strips of dry filter paper were thrust half way down the neck of the Kjeldahl flask and made into a kind of seal after which they were wetted with dilute sulphuric acid. This arrangement also acted as an efficient trap. After the reduction was complete, the papers were thrust into the flask and digested along with the soil. As the filter paper contained no nitrogen and digested very quickly, its inclusion did not in any way affect either the progress of the digestion or the subsequent distillation. Wads of glass-wool soaked in acid and pushed half way down the neck of the Kjeldahl flask also proved efficient in preventing volatilisation of ammonia. In this case, when reduction was complete, the acid in the glass-wool was merely washed down the flask with small quantities of water and the glass-wool removed before digestion.

Some experiments were next conducted with a view to reducing the time needed for reduction of nitrate. The flasks were fitted with the glass-wool traps of the type described above and the contents raised to gentle boiling over a low flame. It was found, however, that owing to condensation of water vapour, the acid in the glass-wool was soon carried down into the flask. As the result of this, the efficiency of absorption was lowered and a part of the ammonia was lost by volatilisation. It was further observed that when the alkali-nitrate mixture was kept warmed at about 50°, the rate of reduction was not very much quickened, at least one hour being needed for completion in most cases. Since even at the laboratory temperature ($25°$–$30°$), the reduction proceeded to completion in $1\frac{1}{2}$ to 2 hours, and since, during that period, the reducing mixture required no attention, it was considered desirable to adopt that procedure.

The estimation of total nitrogen was carried out as follows:— The soil (5 g.) was weighed out into a Kjeldahl flask and treated with 20 c.c. of a 4 per cent. solution of pure potassium hydroxide. Finely divided Devarda's alloy (0.5–1 g.) was then added and the neck of the flask fitted with a wad of glass-wool previously soaked in dilute (5–6 per cent.) sulphuric acid. The mixture was left as such for $1\frac{1}{2}$ to 2 hours, after which period the acid in the glass-wool was washed into the flask by repeated small quantities of water and the glass-wool removed. Concentrated sulphuric acid (20 c.c.) followed by the salt mixture (about 5 g. of K_2SO_4 and 0.5 g. of $CuSO_4.5H_2O$) were then added and the digestion completed as usual. In Table X are given the results obtained according to the above procedure with a few typical soils.

It may be seen that fairly accurate estimates of total nitrogen can be obtained according to the above method. In some cases the soils contained as much as 500 parts per million of nitrate nitrogen, so that the above procedure may be considered to be quite reliable for inclusion of nitrate in the estimate for total nitrogen.

Nitrate reduction followed by oxidative digestion.—With a view to determining whether digestion in presence of oxidising agents can be successfully combined with preliminary reduction of nitrates, the following experiments were carried out. Samples (5 g.) of the Indore and Cuttack soils were treated with known quantities of nitrate and the preliminary reduction in alkaline medium carried out as detailed above. The suspensions were then treated with 20 c.c. each of concentrated sulphuric acid and 5 g. of barium peroxide together with a small quantity (about 0.5 g.) of copper sulphate and the digestions carried out for one hour after the fumes began to appear. In another set of experiments, the soil suspension was treated with 20 c.c. of 6 per cent. hydrogen peroxide together with acid (20 c.c.) and the usual salt

TABLE X.

Soil type		Total N in soil ('Wet' method) p.p.m.	Nitrogen in soil treated with Nitrate		Error per cent. in the estimate of total N
			Expected p.p.m.	Found p.p.m.	
Red loam—Bangalore	..	565·8	939·0	927·0	1·3
Light clay—Sholapur	..	337·2	537·2	532·4	1·0
Light clay—Sholapur	..	337·2	737·2	732·1	0·7
Lowland, paddy—Cuttack	..	767·6	967·6	965·3	0·3
Lowland, paddy—Cuttack	..	767·6	1168·0	1158·0	0·9
Heavy black—Nagpur	..	626·1	812·7	806·7	0·7
Kalar—Sindh	..	722·4	1096·0	1108·0	..
Black cotton—Indore	..	532·5	732·5	730·0	0·4
Black cotton—Indore	..	532·5	932·5	918·5	1·6
Peaty—Travancore	..	5201·0	5574·0	5550·0	0·5
Peaty—Travancore	..	5201·0	5761·0	5731·0	0·9

TABLE XI.

Treatment	Nitrogen in parts per million (Averages)					
	Soil, heavy black, surface—Indore			Soil, Lowland surface—Cuttack		
	Soil alone (control)	Soil+200 p.p.m. of N as nitrate	Soil+400 p.p.m. of N as nitrate	Soil alone (control)	Soil+200 p.p.m. of N as nitrate	Soil+400 p.p.m. of N as nitrate
*H_2O_2—6 per cent. (20 c.c.)—45 mins.	529·3	..	926·4	763·2	958·0	1162·0
BaO_2 (5 g.)—1 hr. ..	534·6	726·8	921·2	761·0	966·4	1149·0
'Wet'—1 hr. ..	516·9	712·2	898·4	732·1	932·1	1146·0
'Wet'—1½ hrs. ..	532·5	730·0	918·5	767·6	965·3	1158·0

* After correction for the nitrogen in hydrogen peroxide.

mixture and the digestion conducted for 45 mins., after the fume-stage. The results thus obtained together with the values for 'wet' digestion for 1 and 1½ hours respectively, have been set forth in Table XI.

It may be seen that digestion in presence of hydrogen peroxide or barium peroxide is complete in a shorter time than that in presence of water alone.

DISCUSSION.

The results of the present enquiry have thrown much useful light on the relative merits of the reduction of nitrates in acid and alkaline media. In either case, ammonia is formed through the intermediary production of nitrous acid or nitrite. But, since nitrous acid is unstable in acid media and since the reduction of nitrate to nitrite would appear to proceed faster than that of nitrite to ammonia, it follows that acid reduction methods involve accumulation of nitrous acid in the medium. Such a condition would result in some loss of nitrogen partly (by decomposition) as nitric oxide and nitrogen peroxide and partly through interaction with soil nitrogenous matter. On the other hand, the alkali nitrite which is formed in the intermediary stages of nitrate reduction in alkaline medium is stable and is not lost in any manner.

When the reduction is conducted in alkaline media at the ordinary temperature, only traces of ammonia tend to volatilise, but even that can be easily trapped by the acidulated glass-wool or filter paper cone inserted in the neck of the flask. During reduction, the Kjeldahl flask is preferably closed with a rubber or wooden stopper, so that none of the ammonia from the atmosphere may get absorbed by the acid trap. No further attention is needed so that, although the reduction takes between 1½ and 2 hours, it involves no strain on the worker.

It would thus seem that reduction in alkaline medium combined with digestion in presence of an oxidising agent is a rapid and highly efficient way of estimating total nitrogen including nitrates. The technique is applicable to all types of soils and there is no correction to be applied except when any of the commercial brands of hydrogen peroxide containing a nitrogenous preservative is used as the oxidiser.

As already indicated, the main drawback about reduction in acid media is the formation of fairly large quantities of nitrous acid as intermediary product. The rate of formation of that acid, as also its subsequent reduction to ammonia, are dependent on a number of factors, the most important among which are probably the nature of the reducing agent and the concentration of the acid employed. The subsequent operations of digestion and distillation would no doubt be greatly facilitated if the products of reaction are soluble in both acid and alkaline media. A systematic enquiry designed to throw light on the above and related factors has been undertaken and will form the subject of a later communication.

SUMMARY.

1.   Preliminary reduction of nitrates in soils by means of ferrous sulphate, zinc or Devarda's alloy in acid media yields fairly accurate values for total nitrogen only when the quantities of nitrate are under 40 parts per million (as nitrogen). When larger quantities are present, the reduction of nitrate is generally incomplete, resulting in low estimates of total nitrogen being obtained.

2.   In acid media, reduction of nitrate is more complete under conditions of 'dry' digestion than under those of 'wet' treatment.

3.   Evidence has been obtained to show that in acid media there is partial loss of nitrogen through interaction between nitrous acid (formed through reduction of nitrate) and the nitrogenous constituents of the soil.   Similar loss of nitrogen takes place when nitrates are reduced in presence of proteins and their degradation products.

4.   The salicylic acid method yields low estimates of total nitrogen (including nitrates) especially in presence of water.

5.   Use of reduced iron in acid medium gives fairly accurate values for total nitrogen, but has certain disadvantages since (a) certain conditions have to be carefully observed during reduction, (b) reduced iron itself contains nitrogen and necessitates performing a blank, (c) large quantities of the reducing agent are usually needed, and (d) distillation is rendered difficult because the precipitated ferric hydroxide causes violent bumping.

6.   Nitrate reduction by Devarda's alloy in presence of 3–4 per cent. alkali proceeds very satisfactorily and is complete at the laboratory temperature (25–30°) in under two hours.   Based on this observation, a simple method of estimating total nitrogen in soils is described.   The method gives correct values for soils containing even upto 500 parts per million of nitrate nitrogen.   Digestion after nitrate reduction can be hastened by addition of oxidising agents like barium or hydrogen peroxide.

7.   The nature of the factors responsible for the incomplete reduction of nitrates and consequent loss of nitrogen from acid media is discussed and future lines of work indicated.