

DETERMINATION OF NITROUS AND NITRIC NITROGEN IN SOILS.

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Although the importance of nitrites and nitrates in the biochemical transformations of the soil and their bearing on the physiology of the plant have been generally recognised, there are yet very few methods for the accurate determination of these two forms of nitrogen. Moreover, the methods usually adopted for the drying, storage and extraction of soil are not adequate for the stability of nitrites, so that the estimates usually obtained are very low. As the result of this, it is generally assumed that the nitrite content of the soil is negligible: indeed, very few workers of the present day make a separate estimation of that form of nitrogen.

Among the commoner methods, the best known and the one most largely used is that of Giess-Ilosvay, a colorimetric method. This is suitable only for the estimation of minute quantities of nitrites. The accuracy of estimation by this method is affected by the presence of organic matter. Among the other methods, those of Lunge, Kubel, Davisson, Robin, Winkler, Cool, and Yoe, Pandalai and Rao¹ are the most satisfactory, but they are not applicable to soils.

Nitrates are generally estimated by the phenol disulphonic method (colorimetric) as modified by Chamot, Pratt and Redfield². The reduction methods of determination are also numerous. Agreeing in principle, they differ from each other chiefly in regard to the reagents employed for reduction. All of them involve the conversion of nitrate to ammonia and the subsequent determination of the ammonia. It is well known that if the soil extract contains any form of organic matter, particularly nitrogenous, there is great chance of these methods giving inaccurate results.

In view of these and the need for more quantitative information regarding the occurrence and distribution of nitrites and nitrates in soils, the present work was taken up.

I. ESTIMATION OF NITRITE.

Stabilization of nitrite in soil samples.

The method of air drying which is generally adopted leads to partial oxidation of nitrite. Storage also leads to low estimates being obtained. It is known that nitrites are unstable in acid media, moderately so under neutral conditions and quite stable in presence of free alkali. Indeed the conversion of nitrite to nitrate in solution is only a question of the pH condition of the medium. The reaction is an autocatalytic one:

$3\text{HNO}_2 = \text{HNO}_3 + 2\text{NO} + \text{H}_2\text{O}$. The nitric oxide absorbs oxygen from the air and renders the reaction continuous. Thus $2\text{NO} + \text{H}_2\text{O} + \text{O} \rightarrow \text{HNO}_2 + \text{HNO}_3$; $2\text{HNO}_2 + \text{O}_2 \rightarrow 2\text{HNO}_3$ and these go on, so that unless the medium is maintained sufficiently alkaline, the deterioration of nitrite is only a question of time. Thus, to ensure the stability of nitrites in the soil it is essential that the medium must be made alkaline. Among the alkalis, caustic soda or potash is useful but it also extracts large quantities of humic matter, which interferes with the subsequent estimations. Sodium carbonate shares the same disadvantage, though to a lesser extent. Caustic lime has been found to be a satisfactory reagent in stabilizing the nitrite present in soil samples. There is no doubt that some small quantity of organic matter is extracted by it, but that can be easily removed by subsequent treatment. In Table I are given the results of lime treatment of fresh soil samples and the results reveal the advantage of such pretreatments in preserving the nitrous nitrogen of soil samples.

TABLE I.

Effect of addition of lime on the stability of nitrite added to the soil

Treatment of soil.	Soil alone.	Soil + 10 p.p.m. of nitrite N.	Soil + 20 p.p.m. of nitrite N.	Soil + 40 p.p.m. of nitrite N.
Nitrite N as estimated after addition of lime	} 6.7	16.7	26.9	46.5
		} 6.9	16.8	26.4
Nitrite N as estimated after standing for one week with lime	} 6.8		16.7	26.8
		} 6.9	16.9	26.8

Clarification and removal of organic matter.

The alkaline extract is sometimes coloured (golden yellow) and must be specially treated to remove interfering organic matter. A number of reagents—alumina cream, charcoal, copper hydroxide, basic lead acetate, calcium carbonate, copper sulphate, potash alum—have been used for this purpose by various workers. A systematic study with several of these reagents in different combinations showed that with caustic lime, copper sulphate and basic lead acetate there was the twofold advantage in that they at once stabilised the entire quantity of the nitrous nitrogen besides clarifying and decolourising the solution. Even in the absence of lead acetate, a clear filtrate was obtained, but the filtration was very slow. The quick filtration combined with the easy washing eliminate the chances of nitrites and nitrates being retained in the precipitate. In Table II are given the results of experiments done with an extract obtained after the above treatment. 50 gm. lots of soils were treated

in each case with nitrite corresponding to 20 p.p.m. and 66 p.p.m. of nitrogen respectively and the nitrite determined by the Griess-Ilosvay colorimetric method.

TABLE II.

Effect of clarifying agents.

Description of soil sample	Clarifying agent	50 gms. soil alone	Soil + 20 p.p.m. of nitrite N.	Soil + 66 p.p.m. of nitrite N.
Bangalore, Sandy loam.	(a) Lime alone	7.6	22.9	68.5
	(b) Lime + Copper sulphate ..	7.6	23.4	73.9
	(c) Lime + Copper sulphate + Basic lead acetate ..	7.6	27.9	74.0
Dry land Soil, Nasik, Bombay.	(a) Lime alone	3.5	24.0	70
	(b) Lime + Copper sulphate ..	3.0	22.9	67
	(c) Lime + Copper sulphate + Basic lead acetate ..	3.2	23.2	69
Pynmacona, Burma.	(a) Lime alone	<i>nil</i>	22	67
	(b) Lime + Copper sulphate ..	<i>nil</i>	22	67
	(c) Lime + Copper sulphate + Basic lead acetate ..	<i>nil</i>	20	66

The efficiency of the copper-lead clarifying mixture was further tested by adding certain forms of organic matter such as glucose and humic acid to the soil and testing the extract for organic carbon. In another set, the soil samples were treated with manures such as farmyard manure, hongay cake, hongay leaf, etc., and the decomposition allowed to proceed under conditions of both dry and wet cultivation. The resulting extracts were also examined for their organic carbon content. It was found, however, that in none of the cases did traces of organic matter pass into the filtrates. The latter were perfectly clear and colourless.

Estimation of nitrite by titration.—Some experiments were next carried out to determine whether the nitrite in solution can be estimated by direct titration. Four methods were tried :

- (i) Direct titration against standard permanganate ;
- (ii) indirect titration against permanganate after addition of excess of acidified hydrogen peroxide ;
- (iii) addition of excess of permanganate and back titrating the unused permanganate against ferrous ammonium sulphate ;
- (iv) addition of excess of permanganate and determining the unused permanganate iodimetrically.

Table III gives the results of these experiments. It may be noted that direct titration against permanganate did not yield accurate results. This confirms the observations of previous workers (Cool and Yoe, *loc. cit.*; Pandalai and Rao, *loc. cit.*)

TABLE III.

Soil and Treatment		Method employed			
		i	ii	iii	iv
Bangalore Soil.	Soil alone	7.8	6.4	6.9	6.5
	Soil + 10 p.p.m. of nitrite N.	18	16.6	17.0	16.6
	Soil + 33 p.p.m. of nitrite N.	42	39.5	40.0	39.7
Peaty soil, Travancore.	Soil alone	30	24.0	25	24.2
	Soil + 10 p.p.m. of nitrite N.	43	34.8	35.9	34.8
	Soil + 33 p.p.m. of nitrite N.	64	63.2	60.0	63.7
Willingdon Farm Pasture plot.	Soil alone	<i>nil</i>	<i>nil</i>	<i>nil</i>	<i>nil</i>
	Soil + 10 p.p.m. of nitrite N.	10.5	10.1	10.2	10.0
	Soil + 33 p.p.m. of nitrite N.	33.8	33.4	33.4	33.2

Addition of excess of acidified hydrogen peroxide and the titration of the unused peroxide proved a very satisfactory method. It must be emphasised, however, that all precautions have to be taken to prevent the deterioration of the hydrogen peroxide solution and to avoid its decomposition by surface catalytic action. Quick titration is another factor to be attended to. The determination of the nitrite by its oxidation by excess of permanganate and the titration of unused permanganate, either by ferrous ammonium sulphate or iodimetrically, also gave satisfactory results, though, in certain cases, the ferrous ammonium sulphate was found to be an unreliable reagent for practical purposes. On the whole, the method (ii) outlined above is very reliable and gives accurate estimates with different kinds of soils.

Oxidation of nitrite by potassium dichromate.—Since dichromate is a more stable reagent than permanganate and since the chances of its reacting with organic matter are small, it was thought worth while to try to use dichromate for oxidising the nitrite in the extract. If a known excess is used, the balance after oxidation can be determined iodimetrically. The results in Table IV show that dichromate is without action on cold nitrite, and that the mixture has to be heated at least to 80°C. before any reaction can commence. Even at higher temperatures, the heating has to be continued for some time before the reaction can proceed to completion.

TABLE IV.

Effect of temperature on the oxidation of nitrite by an acid solution of potassium dichromate.

Heated for 10 minutes at	Nitrite N. as estimated* (in mg.)	Nitrite as estimated† (in mg.)
30°	<i>nil</i>	<i>nil</i>
40°	6.79	12.86
50°	5.03	13.50
60°	6.79	13.89
70°	6.79	13.76
80°	5.27	14.02
90°	5.29	13.50
100°	5.47	13.50
Boiling vigorously	5.29	13.50

It is thus seen that conditions are yet to be standardised under which the oxidation of nitrite by acidified dichromate solution can take place quantitatively. Now, even if it were possible to make the reaction quantitative, there would still have been the inherent difficulty of heating the solution and cooling it, the whole process being very tedious and unsuitable for routine work.

Oxidation of nitrite by hydrogen peroxide.—There are numerous references to show that nitrites are quantitatively oxidised in acid solutions to nitrates by hydrogen peroxide and that the oxidation is instantaneous. Some experiments were done first with pure sodium nitrite solutions and then with soil extracts to which nitrite solutions were added. The solutions were made just acidic and a known excess of the peroxide added (30% Gewichte perhydrol from E. Merck) followed immediately by a large excess of sulphuric acid (1 : 1). The solution was shaken well and forthwith titrated against standard permanganate. The results of a few experiments are given in Table V. The method works very satisfactorily both with pure solutions and soil extracts. A blank determination with all the reagents and with the soil sample should be done. It was found that, by adopting constancy of conditions and careful avoidance of factors which bring about the decomposition of hydrogen peroxide, excellent results of an easily reproducible nature could be got. The only difficulty, however, is in maintaining a stock solution of hydrogen peroxide but it is now well known that a hydrogen peroxide solution in normal sulphuric acid is perfectly stable for 3 weeks. Nevertheless, either freshly prepared solutions or frequent standardisation of stored solutions are desirable as safeguards.

* Expected value, 7.44 mg.

† Expected value, 14.02 mg.

TABLE V.

Source of nitrite nitrogen	Nitrite nitrogen estimated after necessary correction for blank in the case of soils		Percentage oxidation
	Added (in mg.)	Found (in mg.)	
Pure sodium nitrite	6.9	6.9	100
	6.9	6.9	100
	13.8	13.8	100
	13.8	13.5	98
Pure nitrite added to 50 gms. of black cotton soil.	6.9	6.8	98.5
	6.9	6.7	97.1
	13.8	13.2	95.6
	13.8	13.8	100
	13.8	13.8	100
Dry land soil, Nasik, Bombay	6.9	6.8	98.5
	13.8	13.7	99.2
Kari soil, Travancore	6.9	6.4	92.8
	13.8	13.5	98.0
Sandy loam, Bangalore	6.9	6.9	100
	6.9	6.8	98.5
	13.8	13.8	100
	13.8	13.8	100

General procedure for estimation of nitrites.—Based on the foregoing observations, the following procedure has been developed. The soil (50 gms. ; wet or dry) is treated with 30–40 c.c. of caustic lime (50 per cent suspension) and sufficient quantity of water to bring it up to a homogeneous suspension. The latter is well stirred and then treated with 2–4 c.c. of copper sulphate (10 per cent solution) and basic lead acetate (1 to 3 c.c. of a 10 per cent solution) respectively. More water is added if necessary and the suspension again well stirred and filtered through Buchner. The filtrate which should be clear and colourless is then treated with standard permanganate in excess followed by dilute sulphuric acid in excess. The mixture is well shaken and then treated with potassium iodide (2 to 5 c.c. of a 10 per cent solution). The iodine liberated is titrated against standard thiosulphate (0.05 N) in the usual way.

In the case of wet soils, the general procedure will be the same except that the moisture will have to be determined on a separate sample and the calculation made in the usual way.

Nitrite estimation in some Indian soils.—Applying the procedure detailed above, the nitrite content of some representative Indian soils (air dried samples) were determined. The results are presented in Table VI. In each case nitrite nitrogen amounting to 70 p.p.m. was added in the form of sodium nitrite solution and the values given in each case indicate the nitrite nitrogen recovered by the respective methods (after correction for the actual nitrite content of the soils).

TABLE VI

Nitrite contents of some Indian soils

Description of soil	Nitrite (in p.p.m.) as esti- mated by the Griess- Ilosvay method	Nitrite (in p.p.m.) as esti- mated by the new method	Description of soil	Nitrite (in p.p.m.) as esti- mated by the Griess- Ilosvay method	Nitrite (in p.p.m.) as esti- mated by the new method
Red sandy loam, Bangalore ..	69	70	Alluvial soil, Travancore ..	64	70
Dry land soil, Nasik, Bombay	69	69.5	Upland farm, Cuttack ..	69	69.5
Kari soil, Travan- core ..	65	70.4	Pasture Plot, Wellington Farm	69	70
Calcareous soil, N. Bihar ..	69	70	Upland soil, N. Bihar ..	69	69.9
Govt. Wheat Farm, Jacobabad ..	68	68.9	Black cotton soil, Nagpur ..	68	70
Wheat and sugar- cane soil, Punjab	66.4	69.5	Burma, Pynim- cona ..	66	69.5

There was very close agreement between the duplicate values obtained by the new method. The average values (as may be seen from the above) were also higher than those obtained by the Griess-Ilosvay method. In the new method there is the added advantage that the estimation can be carried out with fairly large quantities of soil so that the chances of error through sampling are greatly reduced.

It may be mentioned, however, that in ordinary practice it would be desirable to estimate nitrite on the fresh sample. If the determination cannot be carried out immediately, the soil should be treated with calcium hydroxide and the wet suspension stored as such until the estimation can be taken up. As is well known, drying leads to oxidation of a part of nitrite, so that the estimates as obtained on air dried specimens do not generally convey a correct idea of the original nitrite contents of soils.

Although the procedure adopted for clarification is efficient in removing humic matter and interfering albuminoid substances, it still appeared probable that a few soluble forms of organic matter may pass into the filtrate and interfere with the accuracy of the estimation. Among these, amides and amino

acids may be reasonably expected. In the previous experiments, where freshly decomposing forms of organic matter were added to the soil, it was observed that not even a trace of any organic carbon passed into the filtrate. Experiments with urea and glycine up to a concentration of 10 p.p.m. of nitrogen showed that these substances did not interfere with the estimation. Addition of fairly large amounts, however, did interfere with the accuracy of the estimation, but such quantities do not ordinarily exist in any soil.

II. DETERMINATION OF NITRATE.

Nitrates are generally extracted together with nitrites and, as the extracts are practically free from organic matter, the same samples can also be used for the determination of nitrates. The estimation may be carried out either on separate portions of the extract or on the same sample. In both the cases, the estimate will represent the combined nitrite and nitrate nitrogen. By a separate determination of the former, the latter can be calculated.

In the usual procedure for the estimation of nitrates by titrimetric method, all interfering organic substances have to be destroyed by oxidation. It is generally recognised that destruction of organic matter by reagents such as alkaline permanganate is extremely slow and tedious, prolonged boiling for several hours being required in many cases. The subsequent reduction of nitrate to ammonia especially in alkaline medium is very inconvenient and often requires redistillation. The most efficient reducing agents such as aluminium or Devarda's alloy are so vigorous in their reaction that the alkali is mechanically carried over as spray during distillation. The procedure described below eliminates both the above mentioned defects.

The aqueous extract of soil obtained in the manner described above is treated with caustic alkali solution in excess and boiled for about five minutes, to remove traces of ammonia which may be present. It is then cooled and distilled with Devarda's alloy. The reduction goes on smoothly and to completion. This is traceable to the small quantities of the lead salt originally added as the clarifying agent. A thin coat of lead is formed around the reducing agent and thus mitigates the vigour of the action. Addition of large quantities of lead to the extract will no doubt lead to the formation of thicker coats of lead around the alloy and thus prevent interaction with the alkali, but the small quantities such as are present in the soil extract helps to moderate the reaction without impairing the efficiency.

The above observations are of considerable practical interest. In the usual procedure for the estimation of nitrates by reduction with Devarda's alloy or aluminium, the distillation has to be carried out slowly and with considerable care. Even then it is difficult to prevent traces of alkali being mechanically carried over into the distillate. Addition of small quantity of a lead salt, preferably the acetate (1-3 c.c. of a 10 per cent solution) is highly effective in eliminating this defect. In place of the lead salt any other metal

which can displace aluminium or zinc can be used. The following are some of the results obtained by the modified procedure (Table VII) when applied to a few representative Indian soils. In each case, nitrate nitrogen amounting to 70 p.p.m. was added in the form of a potassium nitrate solution and the values given in the table indicate the nitrate nitrogen recovered in each case by the respective methods (after correction for the actual nitrate contents of the soil).

TABLE VII.

Description of soil sample	NITRATE NITROGEN IN PARTS PER MILLION AS ESTIMATED BY						
	Phenol disulphonic acid method after clarification	Devarda's alloy method	The procedure described in this paper	Description of soil sample	Phenol disulphonic acid method after clarification	Devarda's alloy method	The procedure described in this paper
Dry land soil, Nasik, Bombay	74	73	71	Upland soil, Cuttack	70.07	71	70
	72	74	71		68.80	71	70
Calcareous soil, N. Bihar.	72	73	71	Wellington Farm, Pasture Plot.	71	72	71
	72	72	72		70	73	71
Alluvial soil, Travancore.	70	70	71	Black cotton soil, Nagpur.	69	71	70
	72	73	72				
Red sandy loam, Bangalore.	70	72	71	Khamba village, surface soil, Ahmedabad.	72	73	72
	71	73	71		73	74	72
Kari soil, Travancore	74	76	75	Upland soil, Gorakhpur.	70	71	70
	73	75	75		69	71	70
	75	76	75				
Wheat and sugarcane soil, Punjab.	68	69	67.5	Pyinmeona, Burma	68.9	72	68.9
	68	69	68.5		69.0	74	68.9
				Upland soil, N. Bihar	69.0	70	70.0
					70.0	72	69.8

The advantages of the procedure described above may be enumerated as follows:—(1) The entire quantity of the nitrite present in the soil system can be extracted. (2) The filtration of the extract is very rapid: the filtrate is colourless and completely free from organic matter. (3) The nitrate can be easily estimated by an accurate method of volumetric titration where the chances of error are at a minimum. (4) The extract can be used either as such or after estimation of nitrite for the determination of nitrate as there is no interfering organic matter. (5) The entire procedure including extraction, filtration and the final estimation can be carried out in $1\frac{1}{2}$ to 2 hours so that

with three or four sets of apparatus a large number of determinations can be completed in the course of an average working day.

SUMMARY.

(1) A direct volumetric method for the estimation of nitrite and nitrate in the same sample has been described. By pretreating the soil with a small quantity of slaked lime the nitrite is first stabilised. By adding a clarifying mixture consisting of copper sulphate and lead acetate together with excess of slaked lime, organic matter and other interfering substances are removed. The nitrite in the filtrate is estimated by addition of excess of permanganate or hydrogen peroxide (in acid solution) and back titrating the unused portion.

(2) The procedure can be extended to include nitrates. An aliquot of the aqueous extract is boiled with alkaline permanganate for a few minutes and then distilled with Devarda's alloy or aluminium in the usual way. The small quantity of lead present in the extract forms a light coat around the reducing agent and thus renders the distillation smooth without impairing the efficiency of reduction.

(3) The methods described above have been utilised for the determinations of nitrites and nitrates in a number of tropical soils. It is also eminently suitable for the study of the changes in different seasons and at various stages both before and after manuring. It can be applied to the study of nitrogen transformations in the swamp soil, especially in the earlier stages of puddling when the conditions are favourable for the reduction of nitrate.

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