

AN IMPROVED METHOD OF ESTIMATING NITROGEN IN SOILS AND PLANT MATERIALS.

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(*Read at Symposium, August 29-30, 1936.*)

The importance of the determination of nitrogen, as an item of analytical procedure in agricultural and allied biochemical researches, has been recognised for a long time. In the study of plants, animals or microorganisms; in the evaluation of foods, feeding stuffs, and fertilisers; in the examination of water, sewage and soil, it is an essential operation without which no useful conclusions can be drawn. It will be no exaggeration to state that in pure research alone (apart from routine estimations which are not published) several millions of nitrogen determinations are being carried out annually in different parts of the world.

A considerable amount of attention has, therefore, been devoted, during the past fifty years, to the development of rapid and accurate methods for the determination of nitrogen. Of the several methods that have been proposed, the one originally developed by Kjeldahl (1883) and subsequently modified by Arnold (1887), Gunning (1889) and others, is the best known and has found the most favour with scientific workers. The procedure is, however, slow and tedious, especially when resistant materials of the type of soils, cereal husks and yeasts have to be digested. Besides, it presents a very objectionable feature—the production of acid fumes—necessitating the digestion being conducted in a fume chamber.

Digestion with concentrated sulphuric acid was considered to be quantitative till Bal (1925), working with the Black Cotton soils of the Central Provinces (India), showed that the results thus obtained were low and discordant. He also found that pre-treating the soil with water¹ greatly improved the conditions, facilitating smoother and quicker digestion and yielding higher and more concordant results.

Sreenivasan (1932, 1933, 1934) confirmed Bal's findings and further showed that it applied to other types of soils as well. The low values obtained by 'dry' digestion (with concentrated sulphuric acid) have been shown to be due to the formation of impenetrable protective coats of silica around undigested soil particles. Pre-treatment with water or dilute (1 : 1) acid prevents

¹ Some recent workers (Martin and Griffith, *J.S.C.I.*, 1935, 54, 234T; Walkley, *J. Agric. Sci.*, 1935, 25, 398) have recognised the advantages of pre-treatment with water.

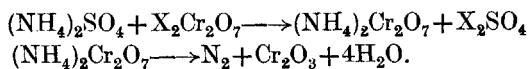
the formation of such coats and facilitates easy penetration, so that the soil is fully digested by the acid. The 'wet' digestion can be greatly accelerated by the addition of small quantities of oxidising agents like hydrogen peroxide.

Marking, as it does, a very important advance in the field of analytical chemistry, still the 'wet' digestion has not got over the chief objectionable feature in the original Kjeldahl method, *viz.*, the emission of acid fumes. It is because of this reason, and the possibility of conducting carbon and nitrogen determinations on the same sample, that the sulphuro-chromic acid digestion has attracted the attention of many workers, especially during the past few years.

The results obtained by some of the previous workers (Anderson and Schutte, 1924; Brown, 1925; Tiurin, 1933; Robertson and Shewan, 1935) with the residue after sulphuro-chromic acid digestion for organic carbon have been very discordant. In view of these divergent results, a critical re-examination of the problem was undertaken by the present authors.

Narayanayya and Subrahmanyan (1935) have shown that direct distillation of the chromic acid digest (such as that left after wet combustion for organic carbon) with alkali gives low values for nitrogen and that better estimates can be obtained by treating the residue with reducing agents such as zinc or reduced iron prior to distillation. They have also shown that chlorides present in the material to be digested cause loss of nitrogen probably due to the interaction of hypochlorous acid and ammonia. The addition of mercuric oxide has been found to overcome this defect. Based on these observations, they have outlined a method for the determination of nitrogen by chromo-sulphuric acid digestion.

The recent researches of Shewan (*loc. cit.*), Acharya (1935, 1936) and the present authors (1935, 1936) have shown that there is loss of nitrogen if the chromo-sulphuric acid digestion is carried out in the usual manner. When the soil is heated continuously with dichromate, water and acid—especially when the initial heating is slow and the digesting mixture is not properly refluxed—the values for nitrogen thus obtained are lower than those secured by the Kjeldahl method. This loss has been traced by us to the intermediary formation and subsequent decomposition of ammonium dichromate:—



This observation is supported by direct experiments with ammonium sulphate and potassium dichromate as also with ammonium dichromate in sulphuric acid media. The formation and subsequent decomposition of ammonium dichromate occur when the potassium dichromate (or chromic anhydride) is added to a cold or moderately hot mixture of soil, acid and water. If, on the other hand, the oxidising agent is added to the boiling mixture of soil and acid and the heating is resumed immediately, the intermediate compound is not formed and, consequently, there is no loss of nitrogen (Table I).

TABLE I.

Effect of addition of dichromate at different stages.

Soil from	Total Nitrogen in parts per million			Expected (Kjeldahl 'wet' digestion)
	Pre-heating to			
	No pre-heating (control)	80°	Boiling	
Tanjore	779	786	793	793
Nilgiris	1722	1767	1818	1816
Kandy	1700	1715	1766	1764

Based on the above observations, we have developed the following procedure for the determination of nitrogen in soils and biological materials. A known amount of the material to be digested is placed in a flat or round-bottomed flask—preferably the one which is to be used subsequently for the distillation—with 2 g. of mercuric oxide (red or yellow variety), 15–20 c.c. of distilled water and 40 c.c. of pure concentrated sulphuric acid. The flask is fitted with an air or water-cooled condenser, and the mixture raised to boil. After boiling for a minute or two, the flame is momentarily removed and 5 g. of potassium dichromate (or the equivalent quantity of chromic acid) are added to the mixture. The heating is resumed immediately and continued for a further period of 30 minutes. The contents of the flask are allowed to cool for about 5 minutes (without removing the condenser), then diluted to about 300 c.c. with distilled water, and reduced by excess of sodium sulphite until a black scum is formed. The mixture is then boiled for a few minutes after which zinc (about 2 g.) is added and the boiling continued for a further period of about 5 minutes. It is then cooled and distilled with excess of alkali in the usual way.

The method has been applied to the determination of the nitrogen contents of various Indian soils and commoner biological materials. The results have been given in Tables II and III.

It has been found (Harihara Iyer and Rajagopalan, 1936) that the estimates obtained by the new method include not only organic nitrogen but also nitrate up to 300 parts per million. Since most soils or biological materials contain only minute quantities of nitrates, the method is quite suitable for the estimation of total nitrogen in such materials. In this connection, the behaviour of zinc as a reducing agent is of special interest. In ordinary acid media zinc reduces nitrates to nitrites, which, on heating, are decomposed resulting in loss of nitrogen (as nitrous fumes— NO_2 and NO) from the medium. After pre-treatment with sulphite, however, and in presence of chromium salts,

TABLE II.

Description of soil	Total Nitrogen in parts per million	
	Expected*	Found
Sind (Willington Cattle Farm)—Alkali soil—green manured ..	520	522
Tanjore (Madras)—deltaic area—alluvial—surface	793	793
Nandyal (Madras)—red clay—surface	296	296
Cuttack (Orissa)—upland—surface	399	402
Gaya (S. Bihar)—lowland, paddy—surface	451	451
Jacobabad—Govt. wheat farm—surface	397	394
Tindivanam (Madras)—dry land—surface	138	138
Gorakhpur—lowland—surface	848	850
N. Bihar—paddy land—surface	643	640
Devarshola (Nilgiris)—estate—surface	1,819	1,821
Kandy (Ceylon)—paddy land—sub-soil	1,769	1,767
Dharwar (Bombay)—old area—sub-soil	537	537
Sholapur—heavy black—surface	244	242
Jaffna (Ceylon)—sub-soil	397	399
Dacca—highland—cultivated—sub-soil	700	702
Chirakkal—sandy loam—surface	315	315
Travancore—alluvial—surface	1,972	1,973
Sind—uncultivated—surface	352	350
Bangalore—gravelly soil—surface	850	850
Nagpur—black cotton—surface	420	422
Mandalay (Burma)—paddy—sub-soil	339	340
Punjab—Barani area—sub-soil	542	542
Ahmedabad—Kambha village—sub-soil	394	397
Chota Nagpur—laterite—sub-soil	209	209
Belgaum—cultivated—sub-soil	466	466

TABLE III.

Material	Total Nitrogen per cent.	
	Expected†	Found
Cyanamide	16.51	16.50
Dried Blood	12.38	12.41
Groundnut cake	7.72	7.68
Lantana leaf	2.80	2.81
Paddy husk	0.27	0.27
Ragi straw	0.52	0.52
Yeast	5.65	5.65

* Including nitrates (Sreenivasan, *J. Ind. Inst. Sci.*, 1935, **18A**, 25).† Sreenivasan, *Ind. Jour. Agric. Soc.*, 1932, **2**, 525.

there is practically no loss of nitrogen. This is traceable to the interaction of hydrogen sulphide (resulting from the reduction of minute quantities of sulphur dioxide left in the medium) with any nitrite that may be formed, yielding hydroxylamine and ammonia.

Hydroxylamine combines with the chromium in the digest and is thus prevented from interaction with any fresh nitrous acid that may be formed. The products are finally reduced quantitatively to ammonia, so that there is no loss of nitrogen from the medium. In Table IV are given some results which will show how added nitrates can be estimated, fairly accurately, by the new method.

TABLE IV.

Description of Soil	Soil alone	Soil + Nitrate (as p.p.m. of nitrogen)					
		50	100	150	200	250	300
Nagpur—Black cotton ..	428	475	520	569	632	680	721
Bangalore—gravelly soil ..	552	600	654	700	749	798	850
Mandalay—alluvial ..	340	387	440	486	544	582	636

A comparative study (Figs. 1 and 2) of the rates of digestion of soils by the different methods shows that the chromo-sulphuric acid digestion is extremely rapid, more than 95 per cent being digested in the first five minutes. The digestion of the remaining part is generally complete in under 20 minutes and in no case is it necessary to continue the heating beyond 30 minutes. Wet digestion, supplemented by hydrogen peroxide, follows next in order, the digestion being complete in $1\frac{1}{2}$ to 2 hours. Pre-treatment with 1:1 acid followed by the usual Kjeldahl digestion is complete in under $2\frac{1}{2}$ hours. The official Kjeldahl (dry) digestion is generally incomplete even at the end of 4 hours. The estimates finally obtained by that method are also lower than those secured by the other methods.

The procedure is quite simple and rapid. As no fumes are evolved, the digestion can be conducted at the working table itself. Given a number of distilling sets, an average worker can complete over two dozen determinations in the course of a working day—an achievement which would be impossible by any of the other known methods.

A further advantage of the chromo-sulphuric acid method lies in the fact that it can be combined with the determination of carbon. We have indeed shown, in a recent communication (1936), how, with a modified type of apparatus, carbonate, organic carbon and total nitrogen can be estimated in the same sample. Such an elegant and rapid procedure is of great advantage, especially in the study of problems relating to the carbon and nitrogen transformations in soils and biological media.

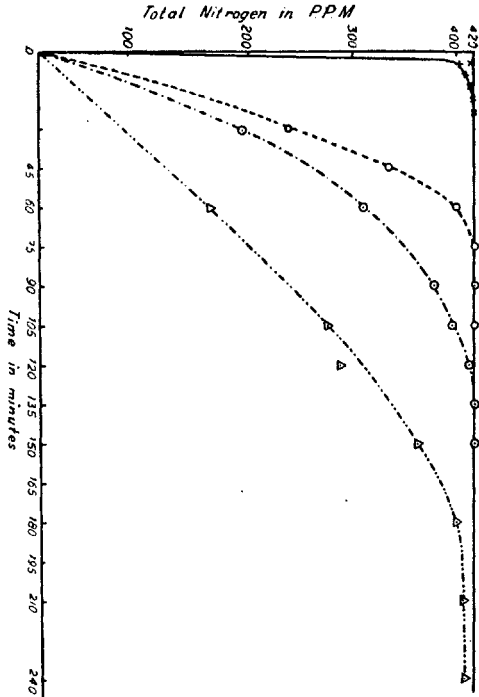


Fig. 1 Rate of digestion of black cotton soil from Nagpur

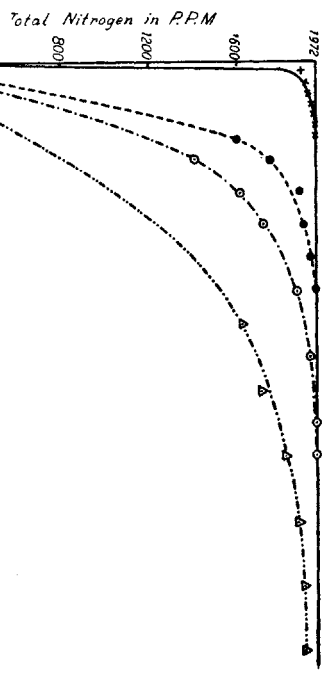


Fig. 2 Rate of digestion of alluvial soil from Trivancore

—•—•— Values obtained by funelless digestion
 —○—○— wet digestion with H_2O
 —●—●— " " " H_2O
 —△—△— dry digestion (official method)

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