

## ESTIMATION OF NITROGEN BY FUMELESS DIGESTION. PART I.

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ESTIMATION of nitrogen is perhaps the most important item of analytical procedure in chemical as well as biological research. In the study of plants, animals or micro-organisms, in the evaluation of foods, feeding stuffs or fertilizers or in the examination of water sewage or soil; determination of nitrogen—in one or more of its several forms—is an essential operation without which no useful conclusions can be drawn. A conservative estimate would, indeed, show that in purely scientific research alone—excluding routine estimations in Government laboratories or in private, consulting or commercial practice for which published records are not generally available—at least a few millions of determinations of nitrogen are being annually carried out by workers in different parts of the world.

Among the various methods for the estimation of nitrogen, the one that is most generally adopted is that originally developed by Kjeldahl (1883) and subsequently modified by several workers (*e.g.*, Arnold, 1887; Gunning, 1889). The procedure is comparatively simple and can be easily followed even in routine operations. It is, on the other hand, slow and tedious, especially when comparatively resistant materials like soils, yeasts, or cereal husks have to be digested. It is also attended by the emission of acid fumes which is perhaps its most objectionable feature.

In recent years, a number of attempts have been made to further simplify the original Kjeldahl method or to hasten the process of digestion. Particular mention should be made of the work of Bal (1925), who showed that addition of water increases the efficiency of digestion. This was followed by the researches of Sreenivasan (1932, 1933 and 1934) who explained the mechanism of the retention of nitrogen during 'dry' digestion and showed that pre-treatment with water combined with small quantities of oxidising agents such as peroxides, dichromates, permanganates or perchlorates, not only hastens the rate of digestion, but also improves the estimate of nitrogen. Valuable contributions have also been made by workers who sought to combine the

wet combustion of carbon with the estimation of nitrogen in the residue (Kruger, 1894 ; Fritsch, 1896 ; Robertson, 1916 ; Anderson and Schutte, 1924 ; Brown, 1927 ; Antipov-Karataiev and Fillipova, 1932 ; Tiurin, 1933 ; Subrahmanyan, Narayanayya and Bhagvat, 1934 ; Robertson and Shewan, 1935 ; Shewan, 1935 ; and Narasimha Acharya, 1935). Their conclusions have been rather discordant, but the recent findings of Narayanayya and Subrahmanyan (1935) would show that, under certain conditions, digestion of nitrogen proceeds to completion in presence of diluted acid and oxidising agents. A simple, fumeless method of estimating nitrogen based on the above principle has been developed and will be described in the present paper.

*Experimental.*

Subrahmanyan, Narayanayya and Bhagvat (*loc. cit.*) have already drawn attention to the fact that the residue left after the wet combustion of carbon gives lower estimates for nitrogen than those obtained by either the official method of 'dry' digestion or the 'wet' procedure described by Sreenivasan and Subrahmanyan (1933). Further experiments with some representative specimens of Indian soils yielded the following results (Table I).

TABLE I.

Description of Soil	Total Nitrogen in parts per million		On the residue after wet combustion of carbon
	As estimated by		
	'Dry' * digestion	'Wet' digestion	
Godavari delta—Alluvial ..	698	717	639
Bangalore—Sandy loam ..	536	566	506
Nagpur—Heavy black ..	639	699	614
Sindh— <i>kalar</i> (alkali) ..	590	604	532
Mandalay—Irrigated rice land	518	536	488
Punjab—Rainfed—Surface..	608	632	578

\* Gunning and Hibbard, "Methods of Analysis," A.O.A.C., 1930.

These observations are in general agreement with those of Robertson (*loc. cit.*) and the more recent findings of Shewan (*loc. cit.*).

*Effect of continuing the digestion after oxidation of carbon.*—Since the ordinary Kjeldahl digestion of soils takes several hours, it appeared probable

that the wet combustion of carbon which occupies less than 30 minutes may not provide sufficient heating which the complete digestion of the different forms of nitrogen may require. With a view to verifying this, some experiments were carried out treating four different specimens of soils (10 g. each) with potassium dichromate (10 g.), water (20 c.c.) and concentrated sulphuric acid (40 c.c.) and digesting them on the sand bath for varying periods of time. The results have been presented in Table II.

TABLE II.

Soil from	Total Nitrogen in parts per million				
	Expected *	After oxidative digestion for			
		30 mins.	60 mins.	90 mins.	120 mins.
Bangalore ..	566	506	506	500	500
Punjab ..	632	578	578	572	575
Godavari delta ..	717	639	642	639	636
Sindh ( <i>kalar</i> ) ..	604	540	532	536	530

\* Sreenivasan and Subrahmanyan, *Indian J. Agric. Sci.*, 1933, 3, 646.

There was considerable difficulty in continuing the digestion after 30 minutes. There was much separation of sand and other mineral matter which caused violent bumping. The results show that even prolonged heating under such conditions led to no improvement in the estimate of nitrogen.

The low estimates of nitrogen obtained in the previous experiments may have been due to (a) retention of nitrogen in some resistant form in either liquid digest or in the insoluble residue, and/or (b) conversion of a portion of the combined nitrogen to nitric acid or gases such as nitrogen peroxide ( $\text{NO}_2$ ), nitric oxide (NO), or even elementary nitrogen. Retention of nitrogen in the digest is known in cases when mercury is used to catalyse the digestion. Sreenivasan and Subrahmanyan (*loc. cit.*) have shown that silica and even increasing quantities of ferric oxide and alumina may retain some nitrogen in the digest. Moreover, the existence of complex ammines, such as those of chromium are known, though their stability in presence of hot, concentrated sulphuric acid and, subsequently, during distillation with concentrated alkali is uncertain. The distillate collected even after prolonged boiling of mixtures

of soil (a specimen from Bangalore), acid and oxidising agent, failed to reveal the presence of either nitric oxide or nitrogen peroxide. Nitric acid was present in no more than traces and this may have been partly derived from the original soil itself. Vigorous heating of mixtures of ammonium sulphate and potassium dichromate or ammonium dichromate alone with sulphuric acid and water failed to show any appreciable loss of nitrogen. The last observation is apparently contradictory to the findings of Shewan, but its significance will be discussed in a subsequent communication. The digestion itself could not be incomplete as, in all the cases, the organic matter was fully oxidised in the course of the first few minutes (Bhagvat, Narayanayya and Subrahmanyam, *loc. cit.*).

*Effect of prolonged heating of the insoluble residue left after digestion.*— Samples (10 g.) of four soils were weighed into a number of Kjeldahl flasks and treated with dichromate (10 g.), water (20 c.c.) and sulphuric acid (40 c.c.). The mixtures were boiled for half an hour to ensure complete oxidation of all organic matter. The digests were then divided into two batches in one of which, after the necessary dilution, the nitrogen contents of the dissolved portion and the insoluble residue were determined separately by distilling with excess of alkali; in the other, the insoluble residue was separated and then treated with water (20 c.c.) and further quantity of sulphuric acid (20 c.c.) and wet digested according to Sreenivasan and Subrahmanyam (*loc. cit.*). The results have been presented in Table III.

TABLE III.

Soil from	Total Nitrogen in parts per million			
	Expected	Found by oxidative digestion		In the residue after further digestion
		In the supernatant	In the residue	
Bangalore ..	566	227	279	279
Sindh ..	722	451	211	211
Mandalay ..	536	421	67	70
Punjab ..	632	482	96	96

The foregoing observations show that the nitrogen retained in the precipitate, if any, is not released on prolonged digestion. The mode of retention would also appear to be of a different type from that recorded by previous

workers (Bal, *loc. cit.*; Sreenivasan and Subrahmanyan, *loc. cit.*) for 'dry' digested soils.

*Effect of chromium sulphate on the efficiency of digestion.*—Since the dichromate ultimately forms potassium and chromium sulphates in the acid medium and since potassium sulphate does not interfere with the progress of digestion, it was considered probable that the formation of the chromium salt may, in some way, be related to the lowering of the estimates. Some experiments were accordingly carried out adding chromium sulphate (10 or 20 g.) in place of the potassium salt to three different types of soils and conducting the Kjeldahl digestion in the usual way. The results have been given in Table IV.

TABLE IV.

Soil from	Total Nitrogen in parts per million		
	With K <sub>2</sub> SO <sub>4</sub> (control)	With Cr <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	
		10 g.	20 g.
Bangalore ..	566	456 ; 500	430 ; 510
Sindh ..	666	529 ; 560	508 ; 572
Godavari Delta ..	717	580 ; 628	600 ; 636

The digestion was rendered very difficult owing to the rapid separation of chromium sesquioxide. There was a tendency to cake at the bottom. Bumping was also rather violent. Owing largely to this, the results of the duplicate determinations were quite discordant. In no case, however, was the value so high as that obtained in the corresponding control.

*Effect of pre-treatment of soil.*—It appeared probable that, as in some of the previous studies, the low values obtained with dichromate may be due to inadequate penetration of the soil by the reagents. Some experiments were accordingly carried out treating the soil-dichromate mixture (10 g. each) with 30 c.c. of water and allowing the suspensions to stand for varying periods of time (4 to 24 hrs.), before adding sulphuric acid. The results which have been given in Table V show that the low values are not due to insufficient penetration, at any rate, of the type described by the previous workers. This conclusion is further supported by the parallel studies of Rajagopal (private communication) on yeast.

TABLE V.

Soil from	Total Nitrogen in parts per million				
	Expected	Oxidative digestion after pre-treatment with water for			
		4 hrs.	8 hrs.	12 hrs.	24 hrs.
Bangalore ..	566	500	510	506	509
Mandalay ..	536	480	490	485	493
Sindh ..	666	550	560	585	565

*Effect of adding chemical precipitants.*—It is well known that the nitrogen retained by mercury salts present in the digest can be released by a suitable precipitant such as potassium sulphide. It was considered probable, therefore, that treatment with certain chemicals either during digestion or just prior to distillation with alkali might lead to the release of any nitrogen that may be held in combination. Some trials were accordingly carried out, the details relating to which, as also the results, have been given in Table VI.

TABLE VI.

Treatment	Total Nitrogen in parts per million	
	Bangalore soil	Sindh soil (2nd sample)
'Wet' (Kjeldahl) digestion (control) ..	566	666
Oxidative digestion .. .. .	506	570
Potassium Sulphide (100 g.) added just prior to distillation .. .. .	500	555
Sodium (ordinary) Phosphate (100 g.) added just prior to distillation ..	496	560
Oxidative digestion with HgSO <sub>4</sub> (15 g.)	398	462
Do. BaSO <sub>4</sub> ( ,, )	500	563
Do. CuSO <sub>4</sub> 5H <sub>2</sub> O ( ,, )	510	566
Do. PbSO <sub>4</sub> ( ,, )	500	563

It may be noted that none of the treatments led to any improvement in the estimate of nitrogen. There was, in fact, marked depression in the case of specimens treated with mercuric sulphate.

*Effect of treatment with reducing agents.*—Samples (10 g.) of three different soils were wet combusted according to Subrahmanyan, Narayanayya and Bhagvat (*loc. cit.*), the digests transferred to distilling flasks and diluted to about 200 c.c. in each case. They were then treated with 10 g. of tin, magnesium or zinc and boiled for 30 minutes. The mixtures were cooled and distilled with excess of alkali in the usual way. Blank determinations were also carried out with the metals alone. The estimates for total nitrogen obtained after making necessary corrections are given in Table VII.

TABLE VII.

Soil from	Total Nitrogen in parts per million				
	Expected	As found by oxidative digestion	Oxidative digestion followed by reduction with		
			Tin	Magnesium	Zinc
Bangalore ..	566	506	520	540	566
Punjab ..	632	578	589	606	635
Mandalay ..	536	488	502	520	540

Tin was comparatively slow in action and was not fully acted on even after 30 minutes of boiling. Magnesium, on the other hand, was very violent in action. The powdered metal tended to float on top and reacted at the surface of the acid with considerable evolution of heat. The effect of that metal as a reducing agent was not, therefore, very pronounced. Zinc was much less violent in its action, but reacted with the acid at a steady rate, and at the end of about 15 minutes, its action was generally complete. The results obtained with that reducing agent were highly satisfactory and corresponded closely to those of the control.

When the above procedure was extended to different types of soils, it was found that correct estimates could not always be obtained. The results varied considerably with the rate of heating. It was found, in consequence, that the volumes of the digests changed with each experiment. As the efficiency of digestion depends, to a large extent, on the constancy of the proportion of acid to water, some experiments were carried out adding water

from time to time to the digesting mixtures and thus maintaining the total volume more or less constant. As this procedure led to greatly improved results, the digestions were next carried out fitting the Kjeldahl flasks with air- or water-cooled condensers. This modification led to accurate estimates being obtained in all the cases. The following results will illustrate the advantages of combining refluxing during digestion with reduction of digest prior to distillation (Table VIII).

TABLE VIII.

Description of soil	Total Nitrogen in parts per million			
	Expected	Found after reduction with zinc alone (digest not refluxed)	Found after refluxing unaccompanied by reduction	Refluxing combined with reduction with zinc
Sindh—( <i>Kalar</i> ) soil ..	572	512	445	572
Nagpur—Black cotton soil	626	572	626	626
Bangalore—Sandy loam ..	566	566	506	566

It may be seen from the above that one soil requires only reduction, while another requires only refluxing. The third requires both the treatments. As the probable behaviour of a soil cannot be easily anticipated, it would be desirable to combine both the treatments in all the cases.

The improvement obtained from refluxing would suggest that, at any rate, in a few cases, the digest contains volatile compounds of nitrogen. Although it was found in some of the earlier experiments that no more than traces of nitric acid were formed from some of the samples that were tried, it is yet probable that others may form that acid in sufficient quantity to affect the estimates of total nitrogen. Moreover, certain types of soils may be naturally rich in nitrates. The nitric acid released on boiling with sulphuric acid may volatilise if the digestion is carried out without proper condensation of the ensuing vapours.

The foregoing observations are in agreement with the recent findings of Narasimha Acharya (*loc. cit.*) and Harihara Iyer and Rajagopalan (private communication). The latter authors have indeed observed that even added nitrates in small quantities can be retained in the digest and included in the estimate of the total nitrogen if the mixture is refluxed during boiling and subsequently reduced in either acid or alkaline medium.

*The mechanism of the action of Zinc.*—The improved estimate resulting from treatment with zinc may be due to (a) reduction of nitrogenous compounds formed in the course of oxidation, and/or (b) dissolution of a part of the precipitate which may otherwise retain some of the nitrogen. The latter is, however, hardly probable since, as already observed, oxidative digestion of yeast yields no precipitate, though the digest has to be treated with zinc before correct estimates of nitrogen can be obtained. The related process seems therefore to be largely one of reduction. As already explained, this conclusion is supported by the presence of minute quantities of nitric acid in the digest. The available evidence is not sufficient, however, to state whether any complex ammine is formed during digestion. The observations with other reducing agents may be summarised as follows. Brass (in the form of powder) acts in acid medium, but is not so effective as zinc alone. Treatment with reduced iron in acid medium yields correct estimates of nitrogen, but the quantity of precipitate formed on addition of alkali is quite considerable so that the subsequent distillation of ammonia is rendered difficult. Aluminium does not act in acid medium, but is very effective in presence of alkali. The latter reaction is, however, very violent, so that there is always the danger of alkali spray being mechanically carried over during the distillation. Similar remarks would also apply to Devarda's alloy, though its action is less violent than that of aluminium. Treatment with milder reducing agents like stannous chloride or oxalic acid does not lead to any marked improvement over the control. It may be concluded from the above that correct estimates of nitrogen can be obtained only by treatment with substances forming nascent hydrogen in either acid or alkaline media.

*Influence of proportion of acid and water on the accuracy of the estimate of nitrogen.*—It was observed that the digestion was comparatively slow when the proportion of acid to water was as 1 : 1 by volume. Even at the end of one hour, the digestion was often incomplete. Increasing the proportion of acid to correspond to the ratio 2 : 1 led to very much more rapid digestion, the entire process being generally complete in about 15 minutes. In no case was it necessary to extend the digestion beyond 30 minutes. Further increase in the proportion of acid to water (4 : 1, 6 : 1 or 8 : 1) did not lead to any corresponding increase in the rate of digestion. The proportion 2 : 1 was therefore adopted in the subsequent studies. In this connection, it may be of interest to mention that Subrahmanyam, Narayanayya and Bhagvat (*loc. cit.*) found the same proportion to be best suited for the wet combustion of carbon.

*Estimation of Nitrogen in soils containing chlorides.*—Anderson and Schutte (*loc. cit.*) have drawn attention to the fact that the estimate of nitrogen

obtained on the residue after wet combustion of carbon is greatly lowered by the presence of chlorides. According to those authors, chlorides react with the ammonia in the digest producing ammonium chloride, which, on interaction with the unused potassium dichromate, evolves gaseous nitrogen through intermediary formation of ammonium dichromate. Our observations have confirmed their findings in regard to reduced estimates being obtained. Indeed, in some of the earlier experiments when concentrated hydrochloric acid or solid sodium chloride was added with a view to reducing the excess of chromic acid in the digest, practically all the nitrogen was lost as gas. On the other hand, the explanation offered by Anderson and Schutte is untenable, because, even ammonia in solution yields elemental nitrogen on treatment with chlorine. Hypochlorous acid which is formed by interaction with water reacts readily with ammonia or amides so that, under such conditions, correct estimates of nitrogen cannot be obtained unless the production of chlorine through interaction between the chloride and chromic acid is avoided.

The previous observations of Subrahmanyam, Narayanayya and Bhagvat (*loc. cit.*) having shown that addition of mercury salts (particularly the oxide and the sulphate) effectively prevents the formation of chlorine under such conditions, some experiments were next carried out with two soils to which known quantities of chloride had been added. The related particulars, as also the results, have been given in Table IX.

TABLE IX.

Soil from	Treatment	Total Nitrogen in parts per million when the digest contains			
		Chloride (0.01 per cent.) as NaCl	Chloride (0.02 per cent.) as NaCl	Chloride (0.05 per cent.) as NaCl	Chloride (0.1 per cent.) as NaCl
Bangalore (Tot. N., 566 p.p.m.)	Without HgSO <sub>4</sub> ..	200	60	16	10
	With HgSO <sub>4</sub> (2 g.) ..	420	407	380	400
	With HgSO <sub>4</sub> + Zinc (2g.)	566	560	566	569
Godavari Delta (Tot. N., 717 p.p.m.)	Without HgSO <sub>4</sub> ..	160	100	18	6
	With HgSO <sub>4</sub> (2 g.) ..	520	500	495	510
	With HgSO <sub>4</sub> + Zinc ..	717	720	717	717

It may be observed that with increasing quantities of chloride, there was correspondingly greater loss of nitrogen until, with 0.1 g., there was practically none left in the digest. Addition of mercuric sulphate was effective in preventing this loss, though, owing to retention of nitrogen, correct estimates could not be obtained, unless the digests were treated with zinc prior to distillation. There was obviously no need to add any chemical precipitant (such as potassium sulphide) to release nitrogen from combination with mercury. This observation is in agreement with the findings of Böttcher (1892) and, more recently, of Harihara Iyer and Rajagopalan (private communication). Even alkali soils rarely contain more than 2 to 3 per cent. of chlorides. Some of the later observations have shown that by addition of a larger quantity of mercuric sulphate (5 g.), loss of nitrogen from specimens containing upto 5 per cent. of chlorides can be prevented. In all the cases, the digest has to be boiled in zinc in the manner previously described.

*Procedure of the estimation of nitrogen in soils.*—Based on the results of the foregoing study, a simple, fumeless method of estimating nitrogen has been developed. The advantages of such a procedure over the usual Kjeldahl method may be enumerated as follows.—The time of digestion is reduced to 30 minutes whereas according to the Kjeldahl method it may often take several hours. Emission of fumes is completely avoided and the digestion can be easily conducted at any working bench in the laboratory. Unlike the Kjeldahl digest which often bumps or otherwise requires frequent attention, the oxidative digestion proceeds smoothly and requires no attention. Addition of mercuric oxide or sulphate may not always be necessary but its inclusion in routine practice is recommended so as to avoid loss of nitrogen, if any of the samples should, unexpectedly, contain chlorides. Reduction with zinc is no doubt an extra operation, but that too requires practically no attention. The reduction proceeds rapidly especially when the zinc is finely powdered and is often complete in under 10 minutes. The distillation proceeds smoothly and takes no more than the usual time. It may thus be reckoned that the entire process of determination—from the weighing of the soil to the back-titration of the unused acid—can be completed in about two hours.

With the exception of the soil, none of the chemicals used in the estimation would require accurate weighing. A convenient procedure would be to weigh out the latter beforehand into a number of packets which may be taken out whenever needed.

*Application of the method to the estimation of nitrogen in some representative Indian soils.*—Following the new procedure, the total nitrogen contents

of some representative specimens of Indian soils were determined. The results, as compared with those obtained by 'wet' digestion, have been given in Table X.

TABLE X.

Locality and description of soil	Total Nitrogen in parts per million as estimated by	
	'Wet' digestion	The new method
Travancore—Loam, alkaline ..	217	219
Sindh— <i>Kalar</i> (saline) ..	572	572
Punjab—Irrigated rice land ..	596	599
Punjab—Rainfed, surface ..	632	629
Nagpur—Heavy, black ..	626	626
Sholapur—Heavy, black ..	243	240
Sholapur—Medium, black ..	415	418
Bangalore—Sandy loam ..	566	566
Ranchi—Upland, surface ..	488	492
South Bihar—Alluvial ..	361	359
Mandalay—Paddy ..	536	536
Jaffna—Sandy ..	325	325

It may be noted that there was close agreement between the two sets of values.

*Modification of procedure to include nitrates.*—Most soils contain only minute quantities of nitrates so that the estimate of total nitrogen is not appreciably affected even if all the nitrate is lost during digestion. There are, nevertheless, certain types of soils which are usually rich in nitrates and which must be specially treated to obtain accurate estimates of total nitrogen. A critical study of the various methods employed for this purpose was recently made by Sreenivasan (1935), who came to the conclusion that reduction of the nitrate with Devarda's alloy in the cold prior to commencement of digestion is the most effective way of including all the nitrate that may be present in the soil system. With a view to determining whether a

similar procedure can be combined with the oxidative digestion of organic nitrogen, known quantities of nitrates were added to specimens of four soils and, after reduction according to Sreenivasan, the total nitrogen contents were estimated in the manner outlined previously. The results, which have been given in Table XI, show that there is close agreement between the values expected and those actually found.

TABLE XI.

Soil from	Total Nitrogen in parts per million		
	Soil alone	Soil+nitrate (200 p.p.m. of N)	Soil+nitrate (400 p.p.m. of N)
Bangalore ..	566	762	960
Ranchi ..	488	680	878
Godavari Delta ..	717	911	1,105
Jaffna ..	325	520	718

Although the procedure adopted in the above experiment yielded accurate results, it was, nevertheless, comparatively slow and tedious. Attempts to hasten the reduction of nitrate by heating proved ineffective, because the resulting ammonia tended to escape through cotton wool soaked in acid which was used as the trap. It was considered necessary, therefore, to adopt a different type of procedure that would first separate the nitrate from the soil and then add it to the digest at the time of distillation. A number of methods were accordingly tried as the result of which it was found that addition of pure calcium sulphate (5 g.) followed by repeated leaching removed all the nitrate but practically no organic matter. Even added nitrates can be successfully removed in this manner. After removal of the nitrate, the soil was digested in the usual manner, and, after reduction with zinc, mixed with the extract containing nitrate and then distilled with excess of alkali and Devarda's alloy (0.5 g.). The results have been given in Table XII.

It was observed that even with 0.5 g. of Devarda's alloy there was considerable frothing and that the alkali tended to pass over with the spray. Addition of a small quantity of paraffin oil reduced the frothing. Metallic zinc was less violent in its action than Devarda's alloy though larger quantities (about 5 g.) had to be used to ensure complete reduction.

TABLE XII.

Description of soil	Total Nitrogen (p.p.m.) as estimated from			
	Soil alone (control)	Soil + nitrate (80 p.p.m. of N)	Soil + nitrate (160 p.p.m. of N)	Soil + nitrate (320 p.p.m. of N)
Travancore—Sandy loam, alkaline . .	217	294	370	530
South Bihar—Alluvial . .	361	435	515	677
Sholapur—Medium, black . .	415	492	570	730
Punjab—Irrigated rice land . .	596	668	750	908

As previously mentioned, most soils may not require any special treatment for the inclusion of nitrates in the estimate of total nitrogen. The more recent observations of Harihara Iyer and Rajagopalan (private communication) would indeed show that no nitric acid is lost during oxidative digestion with refluxing, so that there would appear to be no need for any pre-treatment to include that form of nitrogen.

*Comparative efficiencies of reduction in acid as well as in alkaline media.*—As reduction in acid medium and cooling prior to distillation occupies some time, a number of trials were carried out combining reduction with zinc in alkaline medium together with distillation. The results showed, however, that while correct values were obtained in a few cases, comparatively low estimates were obtained in others. Moreover, when the method was extended to other biological materials such as yeast, leaf powder and seedcake, reduction in alkaline medium yielded lower and less consistent results than that in acid medium.

*Estimation of nitrogen in urea.*—When oxidative digestion was applied, as such, to urea, low and discordant values were obtained. Thus, when a number of parallel specimens were digested, values such as 39.7, 37.6, 40.0 and 38.5 per cents. were obtained instead of the expected value of 46.2 per cent. A study of the related literature showed that, under such conditions, a part of the nitrogen would be lost in the elementary form (Oechner De Coninck, 1899). It was thought probable, however, that by varying the conditions of digestion, such as proportion of acid to water and time of heating, it should be possible to avoid the loss of nitrogen. The results thus obtained have been presented in Table XIII.

Similar low estimates were obtained even when the proportion of acid to water was raised to 6 : 1 : nor was any improvement noticed when the

TABLE XIII.

Proportion of acid to water	Nitrogen per cent. found after digestion for			
	30 mins.	1 hr.	2 hrs.	3 hrs.
2 : 1 ..	38.6	37.8	46.2	38.9
4 : 1 ..	39.5	38.9	39.6	40.5
1 : 1 ..	40.6	39.8	41.2	40.0

solution of urea was pre-treated with alkali for varying periods of time (4-24 hrs.) prior to digestion.

In view of the above difficulty, some experiments were next carried out in which aqueous solutions of urea were boiled with only the acid. It was then found that when the proportion of acid to solution was as 2 : 1, the entire quantity of urea was digested in under half-an-hour.

The observations were then extended to determine whether total nitrogen contents of soils containing urea (and amides in general) can be accurately estimated. To specimens of three soils known quantities of urea were added. The mixtures were first boiled with 2 : 1 acid for 30 mins. and then subjected to oxidative digestion in the usual way. The results have been given in Table XIV.

TABLE XIV.

Soil from	Total Nitrogen (in mg.) as found in			
	Soil alone	Soil+urea (23.1 mg. of N)	Soil+urea (46.2 mg. of N)	Soil+urea (92.4 mg. of N)
Bangalore ..	5.66	28.8	52.0	98.0
Ranchi ..	4.88	28.0	51.0	97.4
Jaffna ..	3.25	26.2	49.5	95.75

It may be noted that very nearly correct results were obtained in all the cases.

The foregoing observations would suggest that if a soil is comparatively rich in free amides, accurate estimates of total nitrogen can be obtained only by pre-digesting soil for about 30 mins. with 2 : 1 acid prior to addition of the

oxidising agent. This would make the process rather long and tedious, so further experiments are in progress to devise a modification that will either eliminate the pre-boiling or, at any rate, reduce it to a minimum. Some encouraging results in this direction have already been obtained and will be reported in the next communication.

*Estimation of nitrogen in cyanamide.*—The difficulty in obtaining accurate estimates of nitrogen in cyanamide is well known. Richardson (1932) who made a special study of this problem has recommended boiling with comparatively dilute acid (acid : water = 2 : 5) for 1–2 hrs. so as to ensure complete hydrolysis of the cyanamide prior to conducting the usual Kjeldahl digestion. Since urea is the immediate product of hydrolysis, it was first thought that adoption of the same procedure as in the previous experiment would yield correct estimates of nitrogen. Such was not, however, the case and, indeed, even longer boiling as suggested by Richardson followed by oxidative digestion failed to yield the correct results. Some further experiments were therefore carried out in which ferrous, ferric or manganous sulphate was added during the pre-boiling (with 2 : 1 acid) stage to catalyse hydrolysis. These yielded the best results and indeed, it was found that in the case of cyanamide alone, the entire digestion was complete after refluxing for about two hours. There was no need for any further oxidation (Table XV).

It may be seen from the above that, except in the last three cases, not only were the values low, but there was also no benefit derived through prolonged boiling. It has to be inferred that in most of those cases nitrogen was either lost from the system or present in some form which was not amenable to oxidative digestion.

When cyanamide (0.2 g.) was added to soil (10 g.) and pre-digested with acid (2 : 1) and manganous sulphate, it was observed that the digestion proceeded very rapidly. There was no need for prolonged boiling as in the case of cyanamide alone. Pre-digestion for 1 hr. followed by the usual oxidative digestion was sufficient to obtain correct estimates of total nitrogen.

The average soil does not contain any free cyanamide. Even if any is added as fertiliser, it is soon decomposed, so that the problem of having to include it in the estimate of total nitrogen rarely ever arises. In cases where cyanamide has been recently added, it can be easily detected and the procedure for the estimation of nitrogen modified accordingly.

*A comprehensive procedure for the estimation of nitrogen in soils and biological media.*—The material to be digested (soil, 10 g. ; others in smaller quantities) is weighed out into a large, flat or round bottom flask, preferably

TABLE XV.

Treatment	Total Nitrogen per cent. after digestion for		
	30 mins.	1 hour	2 hours
2 : 1 acid and $K_2Cr_2O_7$ added simultaneously ..	13.5	13.7	13.6
Refluxed with 2 : 1 acid for 30 mins. and $K_2Cr_2O_7$ then added .. .. .	12.9	13.6	13.0
3 : 2 acid and $K_2Cr_2O_7$ added simultaneously ..	12.8	13.2	12.6
Refluxed with 3 : 2 acid for 30 mins. and $K_2Cr_2O_7$ then added .. .. .	12.6	12.5	12.9
4 : 1 acid and $K_2Cr_2O_7$ added simultaneously ..	11.5	11.8	11.6
1 : 2 acid and $K_2Cr_2O_7$ added simultaneously ..	12.6	12.0	12.4
Overnight standing with 4 per cent. alkali followed by simultaneous addition of excess of acid and $K_2Cr_2O_7$ .. .. .	5.4	..	5.0
Do. $K_2Cr_2O_7$ added after pre-digestion with acid for 30 mins. .. .. .	13.0	..	13.2
Refluxed with 2 : 1 acid and $Fe_2(SO_4)_3$ only ..	14.6	15.4	15.9
„ and $FeSO_4$ only .. .. .	14.6	15.4	15.9
„ and $MnSO_4$ only .. .. .	14.0	15.7	16.1

Value expected=16.3 per cent.

the one to be used subsequently for distillation. Mercuric oxide or sulphate (about 2 g.) is then added and the mixture treated first with water (20 c.c.) and then with pure (N-free), concentrated sulphuric acid (40 c.c.). The flask is fitted with an air-cooled condenser (any piece of glass tube, fairly wide and about 2 feet long will suffice) and the contents raised to gentle boil. After boiling for 30 minutes, potassium dichromate (about 5 g.) is added to the mixture and the boiling continued for a further period of 30 mins. The flask is then taken out and the contents treated with pure, powdered zinc (5-7 g.) followed by dilution with water. This results in very vigorous evolution of hydrogen accompanied by reduction of excess of chromic acid and any nitric acid which may be present in the medium. Any nitrogen

present in combination with chromium is also released by this treatment. The mixture is then boiled for 15 minutes, during which period the added zinc is generally used up. The contents of the flask are then cooled, treated with excess of alkali and distilled in the usual way.

All specimens of zinc—including the purest analytical reagents—contain some nitrogen, so it may be necessary to conduct a blank determination whenever a new sample is taken. A convenient arrangement will be to take a fairly large stock of zinc (1-2 kg.) and to make one set of blank determinations on representative samples taken therefrom.

*Estimation of nitrogen in some biological materials.*—The foregoing procedure was next extended to other organic substances containing free or combined amide nitrogen. In all the cases, the materials to be digested were boiled with manganous sulphate and acid (2 : 1) for 30 mins. prior to addition of oxidising agent. The digestion was then continued for 30 mins. after which the digest was reduced with zinc and distilled with excess of alkali. The results which have been presented in Table XVI show that correct estimates were obtained in all the cases.

TABLE XVI.

Material	Nitrogen (per cent.) as estimated by	
	Kjeldahl ('wet') digestion	Pre-boiling with acid followed by oxidative digestion
Farmyard manure ..	0.64	0.67
Hongay leaf (dry) ..	3.42	3.40
Hongay seed-cake ..	4.48	4.45
Mahua seed-cake ..	2.57	2.48
Dried blood ..	12.53	12.49
Rubber latex—Sample I ..	0.35	0.36
Sample II ..	0.05	0.05

*Experiments with other oxidising agents.*—A few preliminary trials were carried out using chromic anhydride, permanganate and sodium bismuthate in acid as well as alkaline media. The results have been given in Table XVII.

TABLE XVII.

Treatment	Nitrogen in parts per million	
	Expected	Found
Soil (10 g.) + KMnO <sub>4</sub> (10 g.) + NaOH (20 c.c., 50 per cent.). Mixture distilled .. .. .	630	223
Soil (10 g.) + CrO <sub>3</sub> (10 g.) + NaOH (20 c.c., 50 per cent.). Mixture distilled .. .. .	630	102
Soil (10 g.) + Sod. bismuthate (3 g.) + Water (20 c.c.) + H <sub>2</sub> SO <sub>4</sub> (40 c.c.). Mixture digested and then distilled with excess of alkali .. .. .	566	150
Soil (10 g.) + KMnO <sub>4</sub> (20 g.) + Water (20 c.c.) + H <sub>2</sub> SO <sub>4</sub> (40 c.c.). Refluxed for 30 mins. and then distilled with excess of alkali .. .. .	566	340
Do. but reduced with zinc prior to distillation with alkali .. .. .	566	500
Soil (another sample; 10 g.) + KMnO <sub>4</sub> (20 g.) + Water (20 c.c.) + H <sub>2</sub> SO <sub>4</sub> (40 c.c.). Refluxed for 30 mins. and then distilled with excess of alkali .. .. .	630	426
Do. but reduced with zinc prior to distillation with alkali .. .. .	630	581

It was observed that when the soil was distilled as such in alkaline media, there was very slow distillation of ammonia. Even prolonged heating yielded only less than a third of the expected value for nitrogen. Both the bismuthate and the permanganate tended to decompose rapidly in presence of the fairly concentrated acid that was employed. The low values obtained, especially in the case of the former, were largely due to this fact. There was evidence of partial oxidation to nitrate, as also of retention in other forms, in the case of specimens digested with acid permanganate. Further work with more dilute acid solutions and with other oxidising agents is in progress and will be reported in a subsequent communication.

*Discussion.*

The present enquiry has led to a number of findings of much scientific interest. In addition to providing a simple method for the estimation of nitrogen in soils and biological materials, it has also thrown much light on the nature of the changes attendant on the oxidative digestion of nitrogen.

The advantages of the new method have already been enumerated. As the procedure is also comparatively simple, it may be reasonably expected that, before long, it will be adopted in routine practice. A few improvements are, nevertheless, needed. Boiling with the reducing agent in acid medium takes some time and should, if possible, be replaced by some treatment that can be combined with the distillation. A further problem is the blank for the reducing agent which, unfortunately, is highly variable. Even the purest preparations of metals contain some nitrogen, so it may be desirable to reduce the quantity of such agents to the barest possible minimum.

The products of oxidative digestion are more varied than those obtained by the Kjeldahl method. The latter forms exclusively ammonium sulphate whereas the former produces a few other substances as well. During Kjeldahl digestion, there is no loss of nitrogen except that of nitric acid and few other volatile, non-digestible forms which may be originally present, but during oxidative digestion, there is some danger of loss if the conditions are not adequately controlled. There is also the production of nitric acid and certain other complex forms which have to be first reduced if accurate estimates are to be obtained. It is difficult to state whether any nitrogen is mechanically retained under such conditions. Although the new procedure includes all the forms of nitrogen in the estimate, it would, nevertheless, be of much interest to obtain further information regarding the mechanism of the related processes. Such knowledge will also facilitate further simplification of the method of estimating nitrogen.

The difficulties encountered with urea and cyanamide have shown the necessity for modifying the procedure in a few cases. Pre-boiling with acid takes some time and when combined with the oxidative digestion, the process becomes somewhat slow and tedious. There is scope for improvement in this direction and, as previously mentioned, some promising results have already been obtained.

The use of other oxidising agents, though not so far promising, may open out a highly useful field of research. It is probable that some of them may function at much lower concentrations of acid than those required in the case of chromic acid: they may also yield colourless digests either by themselves or after reduction. This would be of great advantage as it would provide a useful check on the progress of digestion.

#### *Summary.*

1. When distilled with excess of alkali, the residue left after wet combustion of soil yielded lower estimates of nitrogen than those obtained by either the official Kjeldahl method or by 'wet' digestion.

2. Prolonged heating of the chromic acid digest led to no appreciable improvement in the estimate of nitrogen: nor was any increase obtained by further 'wet' digestion of the insoluble residue.

3. Kjeldahl digestion with chromium sulphate (in place of potassium sulphate) led to reduced estimates of total nitrogen being obtained. The values were also discordant.

4. Pre-treatment of soil with water did not help to improve the estimate of nitrogen obtained by oxidative digestion. Neither addition of salts of certain heavy metals during digestion nor treatment with certain chemical precipitants before distillation with alkali led to any increase in the value for total nitrogen.

5. Boiling the acid digest with the reducing agents led to marked improvement in the estimate of nitrogen. The best results were obtained with zinc. (All specimens of zinc contain some nitrogen, so some correction must be applied for the ammonia derived from that source.)

6. Some soils yield accurate estimates of total nitrogen even if they are boiled with the oxidising mixture in open Kjeldahl flasks. Others have to be refluxed under air- or water-cooled condensers. A convenient procedure will be to reflux the digest in all the cases and reduce the digest prior to distillation.

7. The mechanism of the action of zinc has been discussed and shown to be largely one of reduction.

8. When the proportion of acid to water was as 1 : 1 or less, the progress of digestion was slow. When the ratio was raised to 2 : 1, it was complete in under 30 minutes. The rate of digestion was not appreciably improved by further increase in the proportion of acid.

9. The presence of chlorides led to marked decrease in the estimate of total nitrogen. This was traced to the formation of chlorine which reacts with ammonia and amides forming elementary nitrogen. The production of chlorine was avoided and correct estimates of total nitrogen obtained by addition of small amounts of mercuric oxide or sulphate to the digesting mixture. The mercury salts in the digest tend to retain some nitrogen but that can be released by boiling with zinc or reduced iron.

10. Based on the above and allied observations, a general method of oxidative digestion applicable to all types of soils has been developed. The procedure for adoption in routine practice has been described. Close agreement has been found between the results obtained by the new method and that determined by Kjeldahl ('wet') digestion.

11. When the soil contains considerable amounts of nitrate, the latter

will have to be reduced with dilute alkali and Devarda's alloy prior to oxidative digestion. An alternative procedure will be to treat the soil with calcium sulphate and water and repeatedly leach out the clear supernatant into the distilling flask. The residue is then subjected to oxidative digestion and the digest, along with the leachate previously obtained, reduced with zinc prior to distillation with alkali.

12. Reduction with zinc in acid medium is more effective than that in alkaline medium. Aluminium or Devarda's alloy is fairly effective in alkaline media, but there is always the danger of alkali being mechanically carried over, thus necessitating re-distillation.

13. Oxidative digestion of urea led invariably to low estimates of total nitrogen being obtained. Neither increase in the proportion of acid to water nor prolonged heating led to any improvement in the estimate. On the other hand, pre-digestion for about 30 minutes with 2 : 1 acid alone was sufficient to digest all the urea. This, combined with oxidative digestion, led to accurate values of total nitrogen in soils containing known amounts of urea.

14. Cyanamide also offered some difficulties, but digestion with 2 : 1 acid containing manganous sulphate hastened the digestion and yielded accurate results. When mixed with soil, the digestion of cyanamide was complete in under one hour.

15. The modified procedure was adopted for the estimation of nitrogen in some biological materials. There was generally close agreement between the values thus obtained and those determined by the Kjeldahl method.

16. The possibility of using other oxidising agents in place of chromic acid has been indicated.

17. Attention has been drawn to the need for further simplification of procedure for oxidative digestion. Certain promising lines of future research have been indicated.

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