Fertilizer Research 19: 39–43, 1989.

Evaluation of distilling the entire digest or an aliquot for total nitrogen determination in soil digests*

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Received 19 April 1988; Accepted in revised form 14 January 1989

Key words: Aliquot of digest, suspension, ^{15}N analysis

Abstract

Accurate and easy to adapt methods of total soil N determination are a prerequisite for N balance research. For ^{15}N balance studies certain modifications of the regular methods are generally adopted, e.g. the distillation of an aliquot of the digest in preference to the entire digest. However, comparative evaluation of such methods has not been investigated. In this study, three methods of distilling soil digests were evaluated for the determination of total N in diverse Alfisols and Vertisols. These are distillation of a clear aliquot (suspended materials allowed to settle) of the digest, distillation of an aliquot with suspended materials, following digestion in a block digestor, and distillation of the entire digest following macro-Kjeldahl digestion. The total N content of soils were determined to be similar whether the aliquot distilled was a clear solution or a suspension with solid materials, and these results were similar to those obtained by distilling the entire digest. The precision obtained by the three methods of distillation was similar for the Vertisols but for the Alfisols, distillation of the clear aliquot of the digest was found to be most precise.

Introduction

Total nitrogen (N) in soils is generally determined using the Kjeldahl method [2]. Most Indian soils have low total N contents, and thus macro-version of the Kjeldahl method is generally preferred for better precision in total N determination. About 10 g sample is needed for the precise determination of total N by the macro-Kjeldahl method.

For N balance studies at our Institute, the samples are digested in 250 ml digestion tubes in a digestion block. An aliquot (50 ml) from the digest made upto 250 ml is distilled with alkali. Nitrogen is then determined by titration after absorbing in boric acid solution [2]. This method is more convenient for preparation of the samples for ^{15}N analysis because it is easily adaptable to an all stainless steel distillation unit [3]. For obtaining a representative aliquot, the digest made upto 250 ml is thoroughly shaken and a 50 ml aliquot of the suspension is quickly drawn using a wide tip pipette.

If the digestion of the soil sample is complete, all of the N should be in solution as ammonium sulfate and there would be no need to take a sample of the suspension. Rather, a clear aliquot after allowing solids to settle should be suitable for analysis. This aspect has not been investigated previously, and the purpose of the work reported in this paper was to (i) compare the results of distillation of the entire digest following macro-Kjeldahl digestion with distillation of an aliquot, and (ii) compare the two methods of obtaining the aliquot i.e. clear aliquot versus suspension, for total N determination in soils with a range in total N contents.

* Submitted as Journal Article No. 776 by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)

Materials and methods

Forty six soil samples were selected to provide a range of samples differing in total N content. The soil samples studied included samples from surface (0—15 cm) and sub-surface horizons (upto 150 cm depth for Vertisols and upto 45 cm depth for Alfisols). The soil samples were ground to pass through a 60-mesh sieve.

The method used for total N determination was a macro-version of the Kjeldahl method modified to include nitrate and nitrite using sodium thiosulfate [4]. In addition to effecting quantitative recovery of nitrate, this method does not require pretreatment of samples with water as is needed in case of the salicylic acid — sodium thiosulfate or $KMnO_4$-Fe modifications of the Kjeldahl method for some soils [1, 5]. Soil samples (10 g) were transferred to 250 ml digestion tubes or 800 ml Kjeldahl flasks and mixed with 20 ml of a 25% aqueous solution of sodium thiosulfate (5 g $Na_2S_2O_3$/ sample), and allowed to stand for 30 min with occasional gentle swirling. Forty ml of concentrated H_2SO_4, and 11.1 g of a digestion mixture consisting of K_2SO_4 + $CuSO_4$. $5H_2O$ + Se in the ratio of 10.0:1.0:0.1 (by weight) was added and the tubes heated gently in a digestion block or on a digestion stand until the water had evaporated and frothing ceased. This was followed by heating the digest at increased temperature (400°C attained in one hour) for one hour after clearing.

The ammonium-N content in the digests was determined by either of three methods: (i) the entire digest was distilled following macro-Kjeldahl digestion. For evaluating the effect of the method of taking digest aliquots, the digests prepared in digestion tubes were made upto 250 ml in the digestion tube and solids were broken with a glass rod and thoroughly mixed by shaking. Fifty ml aliquots were taken from the digests by (ii) quickly withdrawing the aliquot along with suspended material immediately after shaking or by (iii) allowing the digest to settle and then taking a 50 ml aliquot from the supernatant. The ammonium-N in the digest/ aliquot was determined by steam distillation [2].

Table 1. Comparison of the three methods of distilling soil digests for total N determination in Alfisols.[a] Results express total N content (±SE) in mg N kg^{-1} soil

Sl. No.	Method A	Method B	Method C
1	128 ± 2.3	132 ± 0.6	124 ± 0.9
2	223 ± 0.0	226 ± 2.2	219 ± 4.6
3	295 ± 5.2	304 ± 1.9	281 ± 7.2
4	328 ± 2.1	320 ± 3.8	331 ± 1.4
5	368 ± 2.4	351 ± 2.8	358 ± 4.4
6	377 ± 5.0	388 ± 8.5	380 ± 7.3
7	434 ± 5.6	435 ± 6.2	414 ± 3.2
8	448 ± 4.8	440 ± 1.2	422 ± 7.3
9	441 ± 1.8	436 ± 0.3	423 ± 3.9
10	432 ± 3.7	430 ± 4.9	424 ± 5.5
11	459 ± 0.6	456 ± 1.2	444 ± 2.3
12	447 ± 2.0	442 ± 2.1	447 ± 5.1
13	535 ± 0.6	534 ± 3.3	523 ± 3.9
14	550 ± 2.0	546 ± 4.3	538 ± 3.0
15	571 ± 2.1	566 ± 2.2	568 ± 4.9
16	625 ± 4.3	621 ± 1.0	600 ± 3.1
17	609 ± 0.6	602 ± 1.8	601 ± 3.0
18	621 ± 6.8	622 ± 1.5	622 ± 4.6
19	653 ± 9.4	653 ± 3.5	629 ± 1.7
20	645 ± 1.2	641 ± 0.7	634 ± 1.7
21	715 ± 2.2	698 ± 4.9	700 ± 3.8
22	724 ± 3.5	711 ± 2.0	714 ± 3.2
23	740 ± 4.3	725 ± 1.3	718 ± 5.8
24	784 ± 7.8	777 ± 3.6	776 ± 8.1

[a] Method A based on distillation of clear aliquot of the digest, method B based on distillation of aliquot with suspended materials, and method C based on distillation of the entire digest following macro-Kjeldahl digestion.

Table 2. Comparison of the three methods of distilling soil digests for total N determination in Vertisols[a]. Results express total N content (±SE) in mg N kg^{-1} soil

Soil No.	Method A	Method B	Method C
1	204 ± 1.5	206 ± 1.2	199 ± 2.7
2	286 ± 2.5	285 ± 2.3	278 ± 1.2
3	278 ± 3.0	282 ± 1.4	280 ± 0.6
4	317 ± 1.0	315 ± 1.5	320 ± 2.6
5	339 ± 0.9	336 ± 0.7	329 ± 2.0
6	326 ± 2.3	326 ± 1.9	329 ± 0.7
7	352 ± 0.7	348 ± 0.7	336 ± 2.8
8	361 ± 0.3	361 ± 2.9	354 ± 0.3
9	350 ± 5.0	356 ± 2.7	355 ± 1.8
10	386 ± 1.4	388 ± 1.9	375 ± 1.3
11	403 ± 1.7	405 ± 1.5	409 ± 1.5
12	415 ± 0.3	414 ± 2.0	409 ± 0.3
13	419 ± 0.3	420 ± 1.4	418 ± 1.2
14	445 ± 1.4	447 ± 0.0	435 ± 3.8
15	480 ± 0.7	477 ± 1.2	458 ± 6.2
16	501 ± 2.5	500 ± 4.8	486 ± 3.0
17	497 ± 1.0	496 ± 1.2	487 ± 10.8
18	531 ± 1.2	531 ± 3.8	515 ± 1.7
19	557 ± 0.7	559 ± 0.3	547 ± 0.6
20	605 ± 6.0	601 ± 2.4	582 ± 3.1
21	653 ± 0.9	653 ± 3.5	647 ± 1.2
22	1086 ± 3.3	1067 ± 2.0	1067 ± 4.0

[a] Same as for Table 1

Table 3. Comparison of total N content of soils determined using a standard macro and a semimicro Kjeldahl digestion procedure[a]

	Vertisol	Alfisol
No. of samples	16	15
Mean total N *content (%)*		
Macro	0.069	0.061
Semi-micro	0.068	0.061
Mean difference[b]	0.0013 ± 0.00030	0.0003 ± 0.00015

[a] Macro Kjeldahl method involved digestion of the samples on digestion stands and semimicro Kjeldahl method used block digester for digestion. Rest of the procedure was same for the two methods.
[b] Mean of the differences for each sample.

All the analyses reported were done in triplicate and the data were statistically analysed.

Results and discussion

Tables 1 and 2 show that total N contents of the soil digests were similar whether the aliquot distilled was a clear solution or a suspension. It is also clear that the results obtained by aliquot distillation were similar to those obtained using macro-Kjeldahl digestion and distillation of the entire digest. Our earlier work showed that total N values of 31 soils determined using block digesters were in excellent agreement with those obtained using customary digestion stands (Table 3).

Table 4 shows the data on precision of the three methods of distillation and it is clear that the results for total N determination were more precise for Vertisols than Alfisols. This may be due to the fact that the Vertisols used were more homogeneous than the Alfisols. For the Vertisols, all of the three methods of distillation gave similar precision as indicated by SE and CV. However, for the Alfisols, distillation of the clear aliquot of the digest was found to be most precise (Table 4). The correlations between total N values of the pooled 46 soil samples of both Vertisols and Alfisols determined by aliquot distillation (clear or suspension) and by distilling the entire digest were very high (R^2 = 99.8%, Table 5). Also the total N values determined by distilling a clear aliquot of the digest were very highly correlated with those obtained by using an aliquot with suspended materials (R^2 = 99.9%, Table 5).

The results of this study indicate that distillation of a clear aliquot of the soil digest prepared following sodium thiosulfate modification of the Kjeldahl method, after thorough mixing of the digest by shaking and allowing the solids to settle is a precise method and would particularly suit preparation of the samples for ^{15}N analysis.

Comments

As a matter of convenience, we usually prepare digets of a set of soil samples in 250 ml digestion tubes and allow them to stand over night for cooling. Next morning the samples are thoroughly shaken and the volume made to the 250 ml mark. We have observed that about one hour time is sufficient for the suspended materials to settle down at the bottom of the tube leaving a clear digest at the top. A clear aliquot is then drawn from the digest. However, if the digests are to be processed same day, it can be done by following the above described steps once the digests cool to room temperature. Our experience also suggests that the soil digests can be stored for upto 4 days without any change in total N content.

Table 4. Precision of the three methods of distilling the soil digest for total N determination[a]. Results express total N in mg kg^{-1} soil

Method[b]	Alfisol				Vertisol			
	Range	Mean	SE	CV (%)	Range	Mean	SE	CV (%)
A	333–336	335	0.61	0.45	414–419	418	0.76	0.45
B	322–333	326	2.02	1.52	414–417	415	0.52	0.30
C	311–328	322	2.64	2.01	408–412	410	0.61	0.37

[a] Results based on six independent analyses of one soil sample of each soil type.
[b] Method A, distillation of clear aliquot of the digest, method B, distillation of aliquot with suspended materials, and method C, distillation of the entire digest following macro-Kjeldahl digestion.

Table 5. Correlations among the three methods of distilling the soil digest for total N determination. Results are pooled for all soil samples (n = 46)

	Methods compared	R^2 (%)
1.	Clear aliquot (Y) *Vs* entire digest (X)	99.8
2.	Aliquot with suspended material (Y) *Vs* entire digest (X)	99.8
3.	Aliquot with suspended material (Y) *Vs* clear aliquot (X)	99.9

General discussion and review of literature

According to Bremner and Mulvaney [2] the most significant development in the recent years with regard to Kjeldahl digestion has been the use of block digesters for digestion of soil samples. Block digesters have several advantages over the conventional digestion stands. They permit better temperature control during digestion, require less space and allow simultaneous digestion of 20 or 40 samples.

The present method based on the aliquot distillation of soil digest is also very easily adaptable to block digestion. Because in this case we use 250 ml-digestion tubes with mark indicating the volume and therefore after digestion, there is no need for transfering the sample because the volume can be made to 250 ml mark. In the customary digestion, employing digestion stands and Kjeldahl digestion flask, the digest has to be transferred to a volumetric flask, which is very tedious and error could creep in during transfering. Also heating of the samples during digestion using digestion stands could be variable [2]. It is therefore not surprising that the Kjeldahl total N values obtained by using block digesters give slightly higher and more precise values than the similar methods that use customary digestion stands [2, 6].

Another important criterion for N balance studies, is the inclusion of nitrate in the Kjeldahl N. Several methods have been proposed to include nitrate in the total N [2, 4]. A critical evaluation of different methods for inclusion of nitrate and nitrite using ^{15}N labelled compounds showed that the sodium thiosulfate method gives quantitative recovery of nitrate and nitrite [4].

Additionally, the proposed sodium thiosulfate modification of the Kjeldahl N determination [4] does not require additional pretreatment of soil samples with water as does the salicylic acid — sodium thiosulfate or $KMnO_4$-Fe modification of the Kjeldahl method and thus saves considerable time. Additional water pretreatment has been reported to be essential for some soils especially Vertisols with high content of clay for complete digestion of the soil samples [1, 5, 7, 8]. The proposed method [4] was also found to include fixed ammonium in the Kjeldahl N in some soils from Trinidad, West Indies high in fixed ammonium content and there was no need to give any additional HF treatment.

In view of these findings, the proposed method is recommended for total N determiantion in soils as an analyst using this method can analyze a larger number of soil samples because the pretreatments that involve more procedural steps are not necessary.

We have evaluated the aliquot distillation method following digestion of soil samples in block digesters with the conventional macro — Kjeldahl digestion — distillation methods using the salicylic acid — sodium thiosulfate and $KMnO_4$-Fe modifications of the Kjeldahl method for inclusion of nitrate, and the results are in close agreement (data not presented). We have also observed that Kjeldahl methods employing salicylic — sodium thiosulfate or $KMnO_4$-Fe modifications during digestion give digests where solids settle down leaving a clear digest at the top. Separate distillation of the settled solids indicated that they contain very little N and thus are not important for N estimation.

In a recent study, Stumpe et al. [9] suggested that an error could occur from dilution caused by solids that remain following digestion of soil samples using the $KMnO_4$-Fe modification of the Kjeldahl method, when an aliquot is taken from the digest with suspended materials. In fact there are two stages where possible error could be caused by the volume occupied by solids remaining in the digest. The first stage where error could occur is when the digest is made to a certain known volume (250 ml in this study) and solids occupy different volumes. The second stage where error could creep in is when an aliquot is taken with suspended materials especially when different amounts of solids are icluded in the aliquot. The likely error at the first stage would not occur if the volume occupied by solids remaining in the digest following digestion according to

the different modifications of the Kjeldahl method is similar.

Indeed our results with vertisol samples (n = 9) containing more than 50% clay showed that the mean volume occupied by solids remaining using the $KMnO_4$-Fe modification was 24.0 ± 1.00 ml as compared to 19.0 ± 0.44 ml obtained using the sodium thiosulfate modification of the Kjeldahl method. In this experiment 10 g of soil sample was used and the soil digest was made to 250 ml. Thus solids in the $KMnO_4$-Fe method occupied about 2.0% more volume in a 250 ml digest; the increase was contributed by $KMnO_4$ and Fe because the reagent blank digest (without soil) obtained according to this method contained solids which occupied about 5 ml volume as compared to little solids left in the reagent blank digest obtained using the sodium thiosulfate modification.

The possible error at the second stage (while taking aliquot with suspended materials) would be prevented if the amount of solids included in the aliquot with suspended materials is same. In fine-textured clayey soils more suspended materials are likely to be taken along with the aliquot than in light-textured soils. In our opinion, an aliquot taken with suspended materials would always be liable to error because different amounts of solids can be taken along with the aliquot.

However, in the light of our results that solids remaining in the soil digest contribute little to the total N content of a soil sample we suggest that an aliquot be taken from a clear supernatant, after allowing solids to settle at the bottom of the digestion tube. It should however, be ensured that the digest is thoroughly shaken after breaking big lumps, if any in the digest with a glass rod, so that nitrogen if any, retained or trapped by the solids is released into the solution phase.

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