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INCLUSION OF NITRATE AND NITRITE IN THE KJELDAHL NITROGEN  
DETERMINATION OF SOILS AND PLANT MATERIALS USING  
SODIUM THIOSULPHATE

KEY WORDS: Total N, Kjeldahl N, salicylic acid-thiosulphate,  $\text{KMnO}_4$ -Fe, sodium thiosulphate, nitrate, nitrite, soils, plant materials, adenine

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ABSTRACT

Sodium thiosulphate was used in a modification of the regular Kjeldahl method for the determination of total N in soil and plant samples containing  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N. Quantitative recoveries of added  $^{15}\text{N}$ -labelled and unlabelled  $\text{NO}_3^-$ -N (1000  $\mu\text{g}$  N),  $\text{NO}_2^-$ -N (500  $\mu\text{g}$  N), and  $\text{NO}_3^-$ -N (500  $\mu\text{g}$  N) +  $\text{NO}_2^-$ -N (250  $\mu\text{g}$  N) were obtained from soils, plant materials and adenine, even in the presence of water (up to 50 ml  $\text{H}_2\text{O}$ /sample) when 5 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  as 25% aqueous solution was added to each sample before digestion. Moreover, this procedure does not require additional pretreatment of samples as does the salicylic acid-sodium thiosulphate or  $\text{KMnO}_4$ -Fe modification of the Kjeldahl method, and therefore saves considerable time.

INTRODUCTION

Cultivated soils may accumulate significant amounts of nitrate during the fallow period.<sup>1,2</sup> Nitrite is infrequently

present other than in trace amounts but it may increase substantially in alkaline soils after applications of ammonium or fertilizers that react to form ammonium.<sup>3</sup>

The Kjeldahl procedure used to determine total N in soil and plant material must be modified to obtain quantitative recoveries of nitrate ( $\text{NO}_3^-$ -N)<sup>4</sup> and nitrite ( $\text{NO}_2^-$ -N)<sup>4</sup>. These recoveries are important in N balance studies of soil:plant systems to avoid overestimation of losses of N.<sup>5</sup> Bremner<sup>4</sup> listed three such modifications for the analysis of soils: (1) the alkaline reduction<sup>6</sup>, (2) the salicylic acid-sodium thiosulphate,<sup>7</sup> and (3) the permanganate-reduced iron<sup>8</sup> modification.

These modifications including the acidic reduction procedure, recently published<sup>9</sup>, involve additional steps during the analysis to reduce  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N quantitatively to amino-N and ammonium-N which are recovered during the Kjeldahl digestion. These steps are time-consuming and changes to them could improve the efficiency of N analysis.

Bremner<sup>4</sup> stated that  $\text{NO}_3^-$ -N could be quantitatively recovered in the salicylic acid-thiosulphate method if either of these reagents was omitted. Very large amounts of  $\text{NO}_3^-$ -N (58 mg N as  $\text{NaNO}_3$ ), however, were only partially recovered (75%) by 2-3 g of anhydrous sodium thiosulphate when salicylic acid was not used<sup>10</sup> although such large amounts of  $\text{NO}_3^-$ -N are rarely found in soil. Cheng and Bremner<sup>11</sup> observed that salicylic acid was not necessary to get quantitative recovery of  $\text{NO}_2^-$ -N. Pretreatment of samples with thiosulphate only before Kjeldahl digestion may therefore achieve quantitative recovery of  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N. This possibility was tested in the work reported here by pretreating samples of soil and plant material with a solution of thiosulphate and measuring the recovery of added  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N after a Kjeldahl digestion.

## MATERIALS AND METHODS

### Materials

The soils used (Table 1) were surface (0-10 cm) samples obtained from Andhra Pradesh, India (Soils 1-4), Trinidad, West

TABLE 1

Some Characteristics of the Soils

Soil*	Soil series	pH	Clay (%)	Organic C (%)
1	Kasireddipali clay	8.5	54	0.33
2	Kasireddipali clay	8.3	51	0.66
3	Patancheru sandy loam	6.3	19	0.62
4	Patancheru clay loam	6.4	30	0.66
5	Aripo loamy sand	5.7	9	0.30
6	River Estate sandy loam	6.0	18	0.91
7	Maracas clay loam	5.7	39	3.09
8	Cecilvale clay	7.6	45	1.40
9	Langlands-Logie clay	7.1	57	2.17
10	Waco clay	8.3	80	1.75

\*Soils 1 and 2, Typic Pellusterts; Soils 3 and 4, Udic Rhodustalfs; Soil 5, Typic Densiaquults; Soil 6, Fluventic Entropepts; Soil 7, Orthoxic Tropudults; Soils 8 and 9, Udic Chromusterts; and Soil 10, Udic Pellusterts.

Indies (Soils 5-7) and Queensland, Australia (Soils 8-10). The soils varied in pH, clay content, organic C (Table 1) and total N (Table 4). Before use, each sample was air-dried and ground to pass a 2 mm sieve.

The plant materials used were barley straw (Hordeum vulgare) and sunflower stalks (Helianthus annuus) that had been dried at 60°C and ground to pass a 1 mm sieve. Adenine, a heterocyclic organic compound, was also used in  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N recovery experiments to test the efficacy of the proposed  $\text{Na}_2\text{S}_2\text{O}_3$  modification of the Kjeldahl method.

#### Methods

The thiosulphate method adopted after the tests described below was as follows. Samples of 10 g of soil or 0.5 g of plant material were weighed into a 500 ml Kjeldahl digestion flask. The sample was then mixed with 20 ml of a freshly prepared sodium thiosulphate solution (25% aqueous solution, 5 g/sample) and

allowed to stand for 30 minutes with occasional mixing. Then 40 ml of concentrated  $H_2SO_4$  and 11.1 g of a digestion mixture ( $K_2SO_4 + CuSO_4 \cdot 5H_2O + Se$ , 10:1:0.1) were added and the flask heated gently until the water had evaporated and frothing ceased. Heating was then increased and the digests boiled for five hours after they had cleared. The  $NH_4^+$ -N content in the digests was determined by steam distillation as described by Bremner.<sup>4</sup>

The sodium thiosulphate solution was prepared when required, although it may be stored at 4°C for a week without appreciable decomposition. It can also be stabilized by making it slightly alkaline with 1% (w/v)  $Na_2B_4O_7 \cdot 10H_2O$ .

The thiosulphate method described above was tested to ascertain:

1. The effect of varying amounts of thiosulphate,  $NO_3^-$ -N,  $NO_2^-$ -N, and  $NO_3^-$ -N +  $NO_2^-$ -N in aqueous solutions, on the recovery of N (Table 2);

2. The recovery of large additions of  $NO_3^-$ -N and  $NO_2^-$ -N to soil, plant material and adenine (Table 3). (The size of the addition was to overcome the large background of organic nitrogen in the samples);

3. The recovery of small amounts of  $NO_3^-$ -N and  $NO_2^-$ -N added to heavy clay soils (8, 9 and 10 only) using  $^{15}N$ -labelled  $NO_3^-$ -N and  $NO_2^-$ -N as  $Ca(^{15}NO_3)_2$  and  $Na^{15}NO_2$ . (Each had  $^{15}N$  enrichment of 5 atom % added in 10 ml of aqueous solution to provide either 10  $\mu g$  or 100  $\mu g$  added N. After digestion, the  $^{15}N$  in the digest was steam-distilled using stainless steel distillation apparatus.  $^{15}N$  enrichment in the samples was determined by mass spectrometry<sup>12</sup>);

4. The effects of different amounts of water on the recovery of  $NO_3^-$ -N and  $NO_2^-$ -N. (The N was added as 1000  $\mu g$  of  $NO_3^-$ -N or 500  $\mu g$  of  $NO_2^-$ -N, either in 10 ml or 50 ml); and

5. A comparison with the permanganate-reduced iron and the salicylic acid-thiosulphate methods. (In all digests, 1000  $\mu g$  of  $NO_3^-$ -N in 10 ml of water were added to all 10 soils before digestion. To avoid water in the salicylic acid-thiosulphate

TABLE 2

Effect of Amount of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  on  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N Recoveries from Aqueous Solutions

N added ( $\mu\text{g}$ )*		$\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ added (g/sample) <sup>†</sup>				
$\text{NO}_3^-$ -N	$\text{NO}_2^-$ -N	0.5	1.0	2.5	5.0	LSD (P=0.05)
		————— % recovery —————				
100		99	99	100	101	2.5
250		101	100	101	100	2.1
500		95	99	100	100	2.8
1000		78	84	98	99	3.0
1500		65	74	80	85	3.1
2000		41	53	67	75	3.8
	100	99	99	99	101	2.1
	250	98	98	99	100	2.1
	500	90	98	99	99	2.5
	1000		69	75	80	4.8
500	250		99	100	101	2.1
1000	100		80	97	99	2.8
1000	500		69	72	75	3.0

\*10 ml for each sample of aqueous solution of  $\text{KNO}_3$  and/or  $\text{NaNO}_2$ .

†20 ml of aqueous solution of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  for each determination.

digestion, the 10 ml of solution was evaporated to dryness at  $60^\circ\text{C}$  in the digestion flasks before the soil samples were added).

All analyses reported are the means of at least four determinations and the results are expressed on an oven-dry ( $105^\circ\text{C}$ ) weight basis.

### RESULTS AND DISCUSSION

The recovery tests for N in aqueous solutions of  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N demonstrated that the proposed thiosulphate procedure gave a quantitative recovery when 5 g of the reagent was added, and when the amounts of N did not exceed  $1000 \mu\text{g}$   $\text{NO}_3^-$ -N,  $500 \mu\text{g}$   $\text{NO}_2^-$ -N, or  $1000 \mu\text{g}$   $\text{NO}_3^-$ -N plus  $100 \mu\text{g}$   $\text{NO}_2^-$ -N (Table 2).

TABLE 3

Recovery of  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N Added to Soils, Plant Materials and Adenine Using  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in the Proposed Procedure

Sample	Total N $\mu\text{g/g}$	N added ( $\mu\text{g}$ ) <sup>†</sup>				
		$\text{NO}_3^-$ -N		$\text{NO}_2^-$ -N		$(\text{NO}_3^-$ -N+ $\text{NO}_2^-$ -N)
		500	1000	250	500	500+250
————— Recovery of added N (%) —————						
Soil 5	250	99.5±2.2	98.7±2.8	99.8±2.8	98.8±2.3	97.8±3.5
Soil 8	1055	99.2±3.7	98.4±3.4	99.0±2.5	98.5±2.7	98.5±2.5
Soil 9	1831	99.0±3.8	97.5±3.5	99.0±2.8	98.5±2.5	98.7±3.2
Soil 10	1420	98.5±3.8	97.4±3.8	98.9±2.8	97.8±3.5	97.6±4.1
Barley straw	3420	98.5±3.8	95.5±4.5	97.9±4.2	97.0±4.0	97.4±3.9
Sunflower straw	8020	99.3±3.8	98.5±2.7	97.2±4.5	97.0±5.4	96.2±4.0
Adenine	501200	97.8±2.5	96.9±4.1	98.9±2.5	99.5±1.5	97.5±3.7

† 10 ml aqueous solution of  $\text{KNO}_3$  and/or  $\text{NaNO}_2$  was added to each sample of 10 g soil, 0.5 g plant material or 10 mg adenine, followed by 20 ml of 25% aqueous solution of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ . Recovery (%) values are means  $\pm$  s.d. (n = 4).

TABLE 4

Comparison of Total Soil N and the Recovery of  $\text{NO}_3^-$ -N Added to Soils Using  $\text{Na}_2\text{S}_2\text{O}_3$  and Two Other Modifications of the Kjeldahl Method to Include  $\text{NO}_3^-$ -N

Soil	$\text{NO}_3^-$ -N $\mu\text{g/g soil}$	Total N ( $\mu\text{g/g soil}$ )				Recovery of added $\text{NO}_3^-$ -N (%) <sup>†</sup>			
		Salicylic acid-thio sulfate	$\text{KMnO}_4$ -Fe	$\text{Na}_2\text{S}_2\text{O}_3$	LSD ( $P=0.05$ )	Salicylic acid-thio sulfate	$\text{KMnO}_4$ -Fe	$\text{Na}_2\text{S}_2\text{O}_3$	LSD ( $P=0.05$ )
1	14	358	355	352	8	98	99	99	3.2
2	14	565	569	570	6	98	98	99	2.8
3	15	553	556	557	9	99	98	100	3.0
4	15	651	644	649	11	99	97	99	3.8
5	4	246	250	250	5	99	98	98	2.5
6	19	1255	1255	1250	15	99	99	99	1.1
7	25	4150	4198	4270	97	97	97	97	3.5
8	8	1060	1050	1055	18	98	100	98	3.2
9	10	1785	1835	1831	39	97	99	99	3.7
10	5	1398	1420	1419	41	100	97	98	3.5
Mean	13	1202	1213	1220	24	98	98	99	3.0

<sup>†</sup>  $\text{NO}_3^-$ -N was added at the rate of 100  $\mu\text{g/g soil}$ .

The proposed procedure also quantitatively recovered large amounts of  $\text{NO}_3^-$ -N (up to 1000  $\mu\text{g N}$ ),  $\text{NO}_2^-$ -N (up to 500  $\mu\text{g N}$ ), and  $\text{NO}_3^-$ -N (500  $\mu\text{g N}$ ) +  $\text{NO}_2^-$ -N (250  $\mu\text{g N}$ ) when they were added to soils (Table 3). The same was true for small additions of  $^{15}\text{N}$ -labelled  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N. The recoveries of  $^{15}\text{N}$  averaged 100.3% and 102.3%, for the  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N, respectively.

The recovery of  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N by the proposed thiosulphate procedure was not significantly influenced by increasing the amount of water in samples of soil or plant material from 10 to 50 ml (data not presented). The effectiveness of water pretreatment for total N determination in at least some Vertisols when coarse ground samples are used<sup>13,14</sup> increases the desirability of the proposed procedure.

The thiosulphate, the permanaganate reduced iron, and the salicylic acid-thiosulphate procedures all achieved quantitative recovery of  $\text{NO}_3^-$ -N from the ten soils of different physical and chemical characteristics (Table 4). The three methods gave similar total N values for seven of the soils. The salicylic acid-thiosulphate procedure gave low total N values for soils 7, 9 and 10. This is probably because this method does not include a water pretreatment which is known to increase the N values of Kjeldahl digests for some soils.<sup>14</sup>

#### CONCLUSION

The thiosulphate pretreatment procedure that is proposed here gave a quantitative recovery of  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N. It also gave total N contents in soil and plant material similar to those given by two well-established modifications of the Kjeldahl procedure. The thiosulphate pretreatment could therefore replace other pretreatments that involve more procedural steps and therefore more time.

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