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A FIELD METHOD FOR THE DETERMINATION OF TOTAL NITROGEN IN PLANT TISSUE

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ABSTRACT: While studying sward quality in Kenya, East Africa, we established a field laboratory for the determination of total nitrogen. Using Kjeldahl digestion procedures and an indophenol colorimetric method, we successfully analyzed over 900 plant and fecal samples while living in a tent camp. Variation from one digestion to the next was corrected for by using a grass standard with each digestion. Independent tests by other laboratories verified the accuracy of our methods.

INTRODUCTION

The nutritional value of plants can strongly influence plant and animal community structure. As a result, accurate assessment of soil, water, and plant quality is an important component of many ecological studies. Typically, estimates of quality are derived from nitrogen content, not only because of nitrogen's role during plant growth, but because total nitrogen correlates well with many aspects of plant nutrition (eg. crude protein, digestible energy, crude fiber, percent water).

Kjeldahl digestion, which converts nitrogen to ammonium, is probably the most common method of analyzing substances for nitrogen. (1,2,3). These days, with the use of aluminum block digestors (4,5,6,7,8), the process may be carried out more rapidly and at a lower cost than with traditional Kjeldahl digestion apparatus.

Following digestion, the ammonium content in the digest can be estimated by various methods. These include: ammonium detection by an ammonia electrode (9,10,11) steam distillation (12,13), and colorimetric methods (3,6,14,15,16,17,18,19,20,21). AutoAnalyzers, while fast and accurate, are often prohibitively expensive. The above are all considered laboratory procedures.

REAGENTS

Digestion Mix. 7 ml concentrated, low-nitrogen sulfuric acid and one Kjeltab catalyst (3.5 g potassium sulfate, 0.4 g copper sulfate).

EDTA Diluent. Dissolve 6.0 g EDTA·Na_2 in 1000 ml water. Adjust to pH 7.0 with dropwise addition of 1.2 N, low-nitrogen NaOH solution (approx. 15 mL).

1.2 N Sodium Hydroxide. 48 g low-nitrogen NaOH dissolved in 1000 mL water.

Phenol Solution. Dissolve 21.0 g phenol in 300 mL water. Add 0.14 g sodium nitroprusside. Store in an amber bottle at 4° C, and discard upon development of greenish tinge.

Buffered Hypochlorite (pH 11.4-12.0): Dissolve 30.0 g low-N NaOH in 1600 mLwater. Add 100.0 g sodium phosphate. Add 40.0 g sodium citrate. Just before assay, add 5% sodium hypochlorite (Clorox or reagent grade) to buffer (1:4).

Stock Standard: Dissolve 6.0 g ammonium chloride in 100 mL water in a volumetric flask.

External Standard: Bring 0.2 mL of stock standard to volume in a 100 mL volumetric flask.

Note: Phenol, sodium nitroprusside, sodium hypochlorite, and standard solutions should be stored below 4° C.

DIGESTION

The Kjeldahl digestion was performed using a TECATOR block digestor (Model DS-12; capacity: 12 x 100 mL volumetric tubes). In the absence of a fume hood or an exhaust system dependent on running water, the digestion tubes were covered with open-ended, blown glass tubes (a modification on Nelson and Sommers' (4), and Gallaher's (6) funnels). These were serially linked by plastic tubing to augment vapor removal and refluxing (Fig 1). The digestion was performed outside.

Per digestion, approximately 0.3 g (weighed to 0.1 mg on a Sartorius analytical balance, model H51) each of nine grass samples and one SRG were placed into Kjeldahl tubes. A blank of 0.5 mL deionized water was placed in the eleventh tube, and 0.2 mL of stock standard ammonium chloride was added to the final digestion tube as an internal standard. Seven mL of concentrated, low-nitrogen sulfuric acid were added to all tubes. Swirling the tubes ensured saturation with acid, and all samples were left to sit overnight to prevent frothing(22).

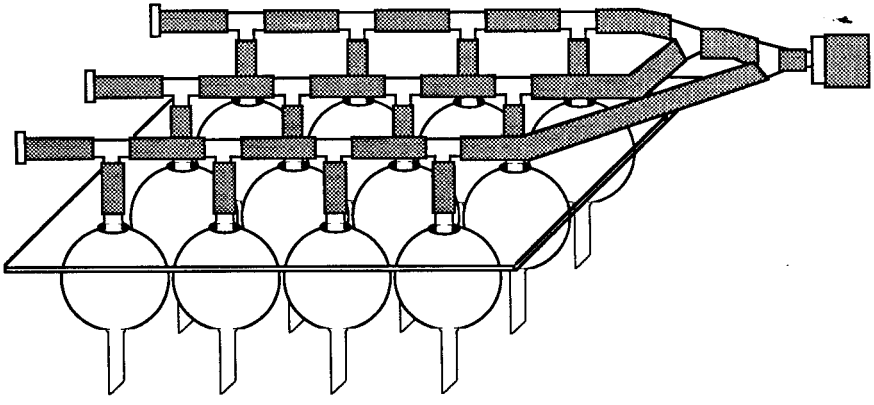

Figure 1. Schematic drawing of apparatus to cover digestion tubes. Blown glass balls are linked by acid resistant tubing within a plexiglass frame. This arrangement facilitates vapor removal and refluxing in the absence of a fumehood.

Following this, one "Kjeltab" catalyst was added to each tube. All tubes were placed in the block digestor at 400° C (1,23). For the first 15 min, the tubes were watched carefully and swirled if frothing began. Boiling chips can be used to prevent bumping though we never experienced this problem.

Samples were digested until they turned from black to a clear emerald color (approximately 1.5 h). The block was then turned off, with the tubes remaining in the hot block another 30 min before being removed. After completely cooling, the tubes were brought to volume with deionized water. Resulting grass digests were then transferred to plastic bottles for storage.

INDOPHENOL NITROGEN DETERMINATION

The following analysis was adapted from Setaro and Jones (21) for use in the field. It was carried out in triplicate using 15 mL polystyrene tubes. All glass and plasticware was washed with 10% hydrochloric acid and rinsed thoroughly with deionized water.

We used a 0.05 mL aliquot of grass digest. An external blank of 0.05 mL of deionized water was used. A 0.05 mL aliquot of the blank digest was used as an internal blank. Internal and external stock standards of 0.25, 0.15, and 0.05 mL were also analyzed. Sodium hydroxide was added, and mixed, to tubes in the following amounts in order to neutralize the digests: 0.2 mL to all grass samples, 0.11 mL to the internal blank, and 0.55, 0.33, and 0.11 mL to the respective internal standard aliquots. Grasses had lost more sulfuric acid during digestion than had the blank or the internal standard. The following reagents were added, one by one and mixed, to all tubes: 4 mL EDTA, 0.5 mL phenol solution, 1.0 mL buffered hypochlorite. These tubes were all mixed, placed in the dark, and the color allowed to develop at room temperature for approximately 7-8 h. The spectral absorbance of each sample was measured using a spectrophotometer (Sequoia-Turner model #340) with a 1-cm cell at 630 nm.

The reliability of this procedure was examined in several ways. To test for within and between digestion effects, three samples each, of three different grasses (the SRG and two others), were digested and analyzed on five seperate occasions. We also analyzed citrus leaves (SRM 1572) of known, but uncertified, nitrogen content, supplied by the Institute of Standards and Technology. Finally, samples were sent to three other laboratories for independent analyses.

CALCULATIONS

The concentration of nitrogen (μg N/mL) in the standard solution was calculated from:

$$5.236 \cdot \text{g } NH_4Cl \cdot (\text{assay vol.}) \div (\text{total tube vol.}) = \mu\text{g N/mL}$$

The three absorbance values from each sample, standard, and blank were averaged, with obviously anomalous values discarded before calculation. This occurred infrequently. A regression was performed to calculate a standard curve of absorbance versus μg N/mL. From the slope, the concentration of nitrogen in the grass samples was calculated. Percent nitrogen was obtained in the following manner:

$$(\mu\text{g N/mL grass}) \div [((500 \cdot (\text{g grass})) \div (\text{total tube vol.})] = \%N$$

TABLE 1.

Results of nitrogen analysis from independent laboratories. Values are % N.

Laboratory	Grass 1 (SRG)	Grass 2	Citrus Leaves
Field lab	2.00	1.09	2.79
U.C.S.B.[a]	1.80	1.10	2.30
Kabete[b]	1.80	1.06	------
S.D.S.U.[c]	1.96	1.03	2.71
N.I.S.T.[d]	------	------	2.86

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[b] Nutrition Lab., University of Nairobi, Box 29053, Kabete, Kenya
[c] Dept. of Biology, Box 43, S.D.S.U. San Diego, CA 92182
[d] National Inst. of Standards and Technology, Gaithersburg, MD 20899

We are confident that these methods provide accurate estimates of nitrogen content. Standard curves generated from internal and external standards were identical throughout the study. 100 digestions of the SRG generated a correlation coefficient of only 5.75. An analysis of the varience from repeated digestions of three grasses revealed that varience arose primarily between digestions (two-way ANOVA of grass and digestion; $F_{2,30} = 687.9$; $P = 0.0001$; and $F_{4,30} = 26.6$; $P = 0.0001$ respectively). Per digestion, varience was highly correlated between grasses (Fig 2). Thus, deviations of the SRG from its mean could be used to correct for between digestion effects on other samples. We followed this procedure to generate our estimates of nitrogen content. These results were similar to those obtained by other laboratories (Table 1).

The similarity between internal and external standards suggests it is unnecessary to run the external standard and blank with each analysis. It should probably be used initially, however, to verify that digestions are proceeding normally. Thereafter, sporadic testing would suffice.

Other researchers (25,26,27) have used sodium salicylate which is found to be more sensitive and subject to less interference by cations than phenol. Baethgen

(27) also preferred this method because it avoided phenol, a carcinogen. We feel their methods could also be adapted to work in the field.

CONCLUSION

The results described in this paper should encourage other workers who wish to analyze nitrogen while living under field conditions. The equipment we used was portable, reliable, and relatively inexpensive. The methods quickly produced accurate estimates of total nitrogen. This rapid feedback improved our study immensely and allowed us to constructively modify our research *in situ.*

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