Spectrophotometric Estimation of Nitrate in Soil Using Chromotropic Acid

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Soil nitrate contents have been measured spectrophotometrically using chromotropic acid. Small volumes (2 ml.) of soil extract are sufficient for the estimation. Nitrate nitrogen contents as low as 0.02 p.p.m. in this extract have been measured, and greater sensitivity can easily be obtained. The method has given quantitative recoveries of up to 100 p.p.m. of nitrate nitrogen added to two soils. Colloidal organic matter and nitrites interfere in the estimation, but both are readily removed. Neither chlorides nor any of the common soil cations interfere at concentrations likely to occur in soil extracts. The technique is relatively simple and rapid.

A SENSITIVE method for determining nitrates in small quantities (1 gram) of soil was required to facilitate studies of nitrate uptake by plant roots. Most techniques require soil samples of at least 10 grams. An exception is the microdiffusion method of Bremner and Shaw (*7*) in which 2-ml. aliquots of soil extract are analyzed. This method proved unsatisfactory, however, because the nitrate could not be completely reduced.

A spectrophotometric method for estimating trace amounts of nitrate in water, described by West and Lyles (*6*), is based on the reaction of nitrate with 4,5-dihydroxy-2,7-naphthalenedisulfonic acid (chromotropic acid). An attempt has been made to adapt this technique to soil analysis. A method has resulted which is rapid, sensitive, and free of serious interferences.

Recommended Procedure

Preparation of Reagents. EXTRACTING SOLUTION. For determining soil nitrate alone prepare a 0.02*N* solution of copper sulfate; if ammonium nitrogen is to be determined in the extract also, add sodium sulfate (1*N*) and sulfuric acid (0.1*N*). If nitrites are suspected or known to occur in the soil, add 0.01% sulfamic acid and acidify to 0.1*N* with sulfuric acid.

CHROMOTROPIC ACID. Prepare a 0.125% stock solution of BDH "for formaldehyde determination" grade chromotropic acid by dissolving the required amount in 6 to 1 sulfuric acid–water (v./v.). Dilute an appropriate volume of this solution daily with dilute (6 to 1) sulfuric acid to give a working solution of 0.0125% concentration. Keep storage bottles stoppered. Prepare fresh stock solutions weekly.

NITRATE STANDARD SOLUTIONS. Prepare standard solutions containing 1, 2, 3, and 4 μg. of nitrogen per ml. by dissolving potassium nitrate in distilled water, with or without 1*N* sodium sulfate and/or 0.01% sulfamic acid. Add sodium sulfate and sulfamic acid, if they are included in the extracting solution.

Analytical Procedure. Add 5 ml. of the extracting solution to each 1 gram of soil in a plastic bottle, and extract for 30 minutes on an end-over-end shaker, or for 2 hours if ammonium nitrogen is also to be estimated. Allow the extract to stand until most of the soil has settled (about 30 minutes). Portions of the supernatant may be removed at this stage for ammonia determination.

CLARIFICATION OF EXTRACT. Transfer 10 ml. of the turbid supernatant to a 10-ml. centrifuge tube. Add 0.2 ml. of either 10*N* or 3*N* sodium hydroxide (to acidified or nonacidified extracts, respectively), and shake thoroughly. Allow to stand for 10 minutes, and then centrifuge to remove the precipitated copper hydroxide and organic matter.

COLOR DEVELOPMENT. If necessary, dilute an aliquot of the clarified extract with water (or sodium sulfate and/or sulfamic acid), so that it contains less than 4 μg. of nitrogen per ml. Pipet 2 ml. of diluted extract into a 6 × 1/2 inch test tube, add 0.3 ml. of 6*N* hydrochloric acid, and mix. Run in 5 ml. of the 0.0125% chromotropic acid reagent, mix thoroughly, and immediately cool to a temperature of less than 20° C. in a water bath. Transfer to a warm water bath (40° C.) for 30 minutes. Recool the tube, and, finally, measure the absorbance of the solution at 362 mμ in a cuvet of 1-cm. light path with distilled water in the reference cuvet.

Standards must be included with each batch of samples.

Observations

West and Lyles added 7 ml. of a 0.01% solution of chromotropic acid in concentrated sulfuric acid to 3 ml. of the nitrate solution, and measured the absorbance of the mixture at a wavelength of 357 mμ after it had cooled at room temperature for 30 minutes. They found it necessary to purify the chromotropic acid (Eastman practical grade) by salting out before use. Sensitivity was increased by adding 1% (v./v.) of concentrated hydrochloric acid to the chromotropic acid reagent. Absorbances were compared with those obtained with standard solutions containing 0 to 5 μg. of nitrate per ml. (0 to 1.13 μg. of nitrogen per ml.). The standard curve showed a marked discontinuity at a nitrate concentration of 1 μg. per ml.

Purity and Composition of Chromotropic Acid Reagent. Chromotropic acid produced by the British Drug Houses, Ltd., and labeled "for formaldehyde determination" was suitable for use without further purification. A reagent concentration of 0.0125% was most satisfactory. The chromotropic acid was dissolved in dilute sulfuric acid (6 to 1 v./v.) instead of in concentrated acid to reduce the temperature reached when reagent and sample were mixed, and hence the possibility of loss of nitrate by volatilization. Lewis (*5*) found that when two volumes of concentrated sulfuric acid were mixed directly with one volume of water, the temperature of the mixture reached 130° C.; if in preparing the same final concentration the concentrated acid was prediluted (5 to 1, v./v.), the maximum temperature was 77° C. The boiling point of nitric acid is 86° C.

Wavelength for Measuring Absorbance. Using the BDH reagent, very high blank backgrounds were recorded at a wavelength of 357 mμ (Figure 1). Satisfactory readings were made at 362 mμ, despite the steep slope of the absorption spectrum at this point. Absorbance should be measured at 362 mμ and standards included with each batch of determinations.

Effect of Chloride on Color Development. The West and Lyles observation that the presence of moderate concentrations of chloride ions increased the sensitivity of the method was confirmed. Maximum sensitivity was achieved when the final chloride concentration was approximately 0.08*N*. At this concentration, however, color development was so rapid that large changes occurred while absorbances were being read (Figure 2); also, the extreme sensitivity unduly restricted the range of soil nitrate contents that could be determined. A final chloride concen-

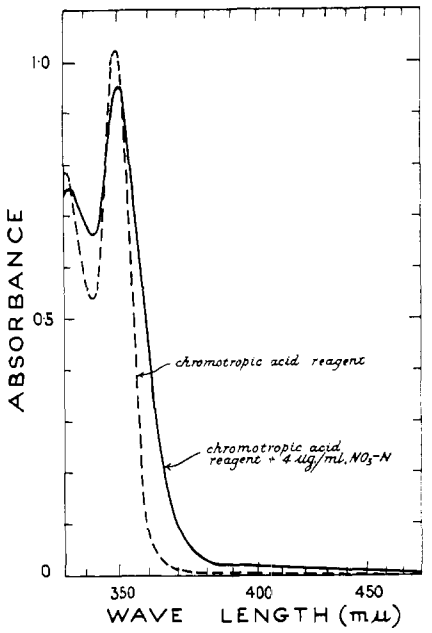

Figure 1. Absorption spectra for chromotropic acid reagent with and without added nitrate

Using 5-mm. spectrophotometer cuvet

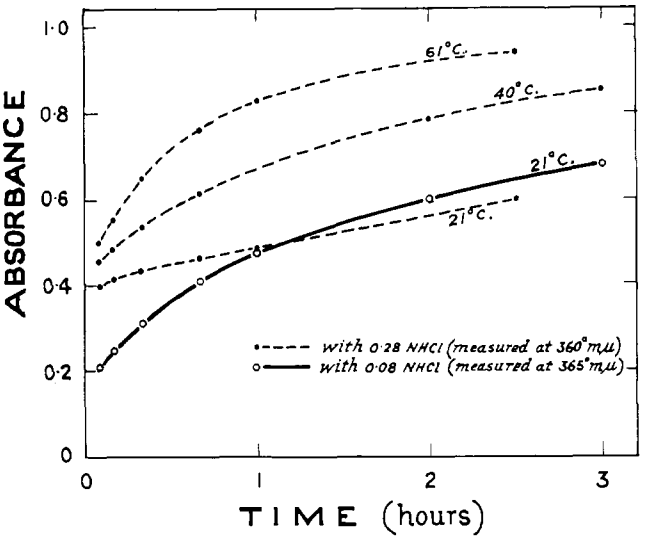

Figure 2. Effects of time, temperature, and chloride concentration on color development curves for chromotropic acid reagent plus 2 µg. of nitrate nitrogen per ml.

tration of 0.25*N* was satisfactory. At this level soil chlorides have negligible effects on nitrate estimations, a soil concentration of 1000 p.p.m. causing an error of only 0.05 p.p.m. of nitrate nitrogen. The required amount of 6*N* hydrochloric acid was added to each aliquot of nitrate solution before addition of the chromotropic acid reagent.

Rate of Color Development. Contrary to the West and Lyles data, it was found that full color did not develop for several hours and that the rate of development depended on the ambient temperature (Figure 2).

Satisfactory color developed in 30 minutes when the mixture was held at 40° C. in a water bath. Color development was largely restricted to this period by cooling the mixture briefly, both before and after immersing it in the warm water.

Range and Sensitivity of Nitrate Estimations. Using 1 to 5 soil-solution extracts, soil nitrate nitrogen levels ranging from 0.1 to 20 p.p.m. were measured, and when required the upper limit was extended by dilution. The standard error of six determinations made at 0.11 p.p.m. was 0.02 p.p.m., and that of 12 made at 0.33 p.p.m. was 0.05 p.p.m. Greater sensitivity can be achieved if necessary by reducing the amount of hydrochloric acid added, or by increasing the concentration of the chromotropic acid reagent, the ambient temperature during color development, or the optical path of the spectrophotometer cuvet.

The standard curve was almost linear over the full concentration range used (Figure 3).

Potential Interferences. Organic Matter. Soil extracts must be completely clarified before chromotropic acid is added, and in particular colloidal organic matter must be removed. This was done satisfactorily, by precipitating copper hydroxide from the extracts as recommended by Harper (*3*) and Lewis (*4*). The copper hydroxide treatment did not interfere in the determination.

Soluble components of the soil extract, not removed by this treatment—presumably soluble organic material—may interfere in the determination by reacting with the strong sulfuric acid in the reagent. The magnitude of this interference was assessed by adding reagent strength sulfuric acid to either distilled water or clarified extracts of three soils; measuring absorbances were obtained. The interferences, expressed as equivalent increases in nitrate nitrogen values, are shown in Table I. This reaction is unlikely to produce large errors in soil nitrate determinations.

Soluble soil organic materials may also interfere by reacting with the chromotropic acid. This reaction cannot be a serious source of error with the Urrbrae and Wanbi soils, however, as nitrate nitrogen values as low as 0.3 and 0.1 p.p.m., respectively, have been obtained with these soils, and nitrate recoveries have been complete. Other soils have not been studied.

Cations. Cations which may be extracted from soils in significant amounts by acid extracting solutions include Ca^{+2}, Mg^{+2}, NH_4^+, K^+, Na^+, Fe^{+3}, and Al^{+3}. Fe^{+3} is precipitated as $Fe(OH)_3$ on the addition of alkali, and so is removed when the extract is clarified with copper hydroxide. Solutions of the remaining cations were added as chlorides to a standard nitrate solution (replacing an appropriate quantity of HCl), to determine their effect on nitrate estimation. When added at rates approximately maximal for soil extracts, no changes in measured absorbances were produced. Much higher concentrations of Na^+ or K^+, however, caused slight decreases in absorbance; therefore, if sodium or potassium salts are used in extracting solutions, appropriate amounts should be added to standard nitrate solutions.

Nitrites. Nitrites rarely accumulate in soils in significant amounts (*2*), but nevertheless should be considered as a possible source of interference. West and Lyles reported that they interfered quantitatively in the nitrate–chromotropic acid reaction. In the present investigations increases in absorbance due to nitrite were 44% as great as those caused by nitrate at equivalent concentrations.

Bremner and Shaw (*1*) removed nitrites by adding 0.2% sulfamic acid to their acidic extracting solutions. Applied to the chromotropic acid procedure this treatment eliminated nitrite interference, but halved the sensitivity of the nitrate determination. Absorption spectra studies showed that this loss of sensitivity was due to a reaction between the chromotropic and sulfamic acids, and could be overcome by reducing the concentration of sulfamic acid. Thus 0.01% sulfamic acid in the extracting solution completely removed 4 p.p.m. of solution nitrite nitrogen, but scarcely affected the nitrate estimation.

Nitrite interference can therefore be readily overcome at soil concentrations

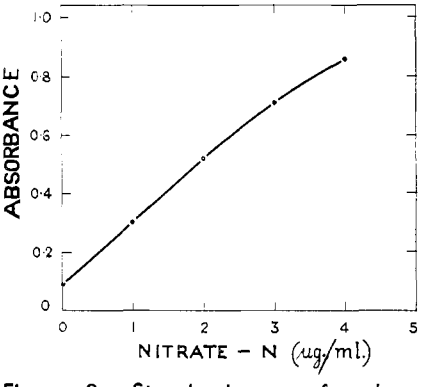

Figure 3. Standard curve for chromotropic acid reagent plus nitrate nitrogen

Table I. Interferences Due to Reaction of Soluble Soil Extract Components with Sulfuric Acid, Expressed as Equivalent Increases in Soil Nitrate Values

Soil	Organic C Content of Soil, %	Interference, P.P.M. NO_3-N
Urrbrae loam (red-brown earth)	2.2	0.20
Claremont clay (Wiesenboden)	4.2	0.04
Wollongbar clay loam (Krasnozem)	5.8	0.47

less than 20 p.p.m. by adding 0.01% sulfamic acid to extractants and standard nitrate solutions. Also, to ensure rapid removal of nitrite the extracting solution should be acidified to pH 1 with sulfuric acid. At higher nitrite levels, which require larger additions of sulfamic acid, some adjustment of technique may be needed to maintain a desired level of sensitivity in the nitrate estimation.

Table II. Recovery of Nitrate Nitrogen Added to Sterile Soil

Soil	Nitrate N, P.P.M. Added	Recovered
Urrbrae loam	35.4	101.2
	42.0	100.1
	66.3	100.0
	92.4	98.2
	100.2	99.9
Wanbi sand	16.3	99.7
	20.0	98.8
	40.0	101.5
	50.4	97.7

Recovery of Nitrate Added to Soil. Known amounts of nitrate added to soil which had been leached and sterilized by γ-irradiation, were recovered quantitatively (Table II).

Chromotropic Acid–Nitrate Reaction

The nature of this reaction is not known with certainty. West and Lyles (*6*) believed that a nitro derivative of chromotropic acid was formed, in a manner similar to the nitration of phenoldisulfonic acid. More than one product may be formed with some samples of chromotropic acid. Thus the West and Lyles reagent gave absorption spectra with a pronounced "hump" at wavelengths of 400 to 450 mμ in addition to a peak at 357 mμ, whereas with the BDH reagent this second maximum was lacking and the main peak was displaced to approximately 350 mμ (Figure 1). The role of chloride ions in increasing the intensity of color development is also unknown.

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NITROGEN AVAILABILITY

Nitrification of Fractions from Commercial Ureaforms

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Three commercial ureaforms have been fractionated by use of the cold and hot water solubility procedures used in the activity index determination. Approximately equal fractions—cold water–soluble, cold water–insoluble but hot water–soluble, and hot water–insoluble—were nitrified at rapid, intermediate, and slow rates, respectively. Good agreement was obtained in comparing nitrification rates of these ureaforms with the values obtained by recombination of their fractions, showing that the fractions were not greatly changed by the fractionation procedure and had no significant effect on each other in nitrification. The effect of granule size on nitrification rate is much less for commercial ureaforms than for other insoluble nitrogen sources such as oxamide or magnesium ammonium phosphate. While affected by granule size to some extent, ureaforms show their characteristic nitrogen release pattern, even when finely divided.

UREAFORM fertilizers are generally considered to consist of a continuous series of polymethyleneureas, as shown by the increase in solubility and in rate of nitrification observed as the urea-formaldehyde mole ratio is increased and the average molecular weight is decreased (*6, 20*). Rates of nitrification of ureaforms were originally related to solubility through the activity index (AI) (*9, 17*), and the general nitrification pattern for ureaforms has been confirmed by subsequent work (*3, 5*).

While it has not been possible to separate the individual components from a ureaform, there have been several studies on nitrification of the lower methyleneureas (mono-, di-, and tri-) and on related fractions (*15, 16, 19*). These methyleneureas all nitrified rapidly, while somewhat higher fractions gave much slower rates.

Long and Volk (*16*) reported nitrification studies on the insoluble portions from ureaform types derived from both solids and solutions. Similar data on the insoluble portion of a commercial ureaform have been reported in the trade literature (*14*). Pereira and Smith (*18*) separated commercial ureaforms into four fractions based on the solubility determinations of the activity index procedure. However, their nitrification studies were confined to two fractions, and while the rates observed showed the greater availability of the more soluble materials, the quantitative relationships were inconclusive.

This paper presents the results of a similar but somewhat more comprehensive study on nitrification of fractions from commercial ureaforms, which was in progress when the work of Pereira and Smith was first reported.

While the nitrification studies on ureaform fractions were run on samples ground according to a standardized procedure, the effect of granule size on the availability of fertilizers has been shown to be closely related to the surface area of the granules (*2, 11, 12*). This effect has been studied in nitrogen fertilizers, particularly for oxamide (*11*) and magnesium ammonium phosphate