

CONVENIENT ESTIMATION OF SOIL ORGANIC-N  
USING THE UDY DYE SYSTEM

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ABSTRACT

A method was developed for conveniently estimating organic-N in soils using Udy dye, which is bound on the positive sites of the soil organic matter complex. The quantity bound is associated with the quantity of organic-N. These amounts can be determined by initially adding an excess of dye and, after equilibrium, colorimetrically measuring the amount remaining in solution. A soil pretreatment wash with water and oxalic acid is used to remove soil solution coloration, buffer the soil pH, and precipitate soluble soil calcium. High amounts of soil free iron oxides or carbonates interfered with the test.

INTRODUCTION

Among the uses made from soil organic carbon analysis is that of estimating soil organic nitrogen content. This

interpretation is based on the ratio of C:N being constant at about 10:1.<sup>1</sup> If a more precise measurement of nitrogen is needed, then the Kjeldahl method is normally used.

With plant and animal products, the Udy dye method<sup>2,3,4,5</sup> is commonly used as an alternative to the Kjeldahl procedure as it is rapid and accurate. The Udy dye system utilizes a monosulfonic azo dye, Acid Orange 12, which is bound on the positive adsorptive sites of protein when the dye solution and protein material is mixed. A decrease in solution dye concentration is correlated with protein content. The dye solution also contains a microbial inhibitor and a buffer system to maintain proper pH and retain color longevity.

This paper presents a method of using the Udy dye system for estimating soil organic-N.

#### MATERIALS AND APPARATUS

Oxalic acid - dry crystal form

Udy dye - a buffered Acid Orange No. 12 azo dye  
Stock No. SL-1215, Udy Analyzer Co.,  
P. O. Box 148, Boulder, Colorado 80302<sup>6</sup>

Centrifuge

Colorimeter with 482 nm wavelength setting or filter

#### PROPOSED METHOD

Grind the soil to pass through a 0.5 mm sieve. Weigh 1 g soil into a centrifuge tube. Add 0.5 g dry crystal oxalic acid and 25 ml water. Cap and shake for 15 minutes on machine shaker at a speed fast enough to maintain the soil in a suspended state, or hand shake every 5 minutes over one half-hour period. Centri-

fuge soil down (RFC = 1000, until supernatant liquid is clear) and pour off and discard supernatant liquid. Add 5 ml Udy dye solution (initially diluted concentrate as per manufacturer's recommendations.) Dye solution temperatures throughout the test should be maintained constant in accordance with manufacturer's recommendations, such as at room temperature. Cap and shake for 15 minutes on machine shaker (or hand shake every 5 minutes for one-half hour) and centrifuge. Pipette 1.0 ml of supernatant dye out of centrifuge tube into a 100-ml graduated cylinder. Add 79 ml water for a total of 80 ml solution for colorimeter reading.

Two reference solutions are needed: First, make a minus 0.025 %N reference by pipetting 1.0 ml Udy dye into a 100-ml graduated cylinder and adding 79 ml water (this is not a 0 %N reference as might be expected, because there must be compensation for the diluting effect of wash water remaining in the soil sample after decanting). Next, make a 0.175 %N reference by taking a 25-ml aliquot of the minus 0.025 %N reference and dilute it with 25 ml of water.

With the colorimeter set at 482 nm wavelength (or filter) to give maximum light absorbance, set the colorimeter at 0% transmittance with cuvette removed (light shutter closed). Then with a water sample in place, set on 0 absorbance (100% transmittance).

To calibrate, measure absorbance readings for the minus 0.025 and 0.175 %N references and record. On graph paper, mark off absorbance values from about 0 to 1.2 on the abscissa, and %N values ranging from minus 0.025 to 0.325 on the ordinate axis. Plot the

two reference points relative to absorbance and %N and draw a straight line through these two points and extending over the range of the graph. The straight line relationship results from the dye's conforming to Beer's law (absorbance is proportional to concentration). This aspect allows intermediate %N references to be optionally made for confirming the calibration. Finally, when an absorbance value of a soil extract is determined, the soil %N can be read from the graph.

An alternate method of determining the %N of unknowns would be to mathematically compute the formula of the line and evaluate it at any given absorbance. Still another related method utilized the formal definition of absorbance (= minus log transmittance), which allows % transmittance to be used. Here the % transmittance values are plotted on the log scale of semilog paper vs %N on the linear scale, and the calibration appears as a straight line which passes through the reference points.

For soils with greater than 0.3 %N, the size of the soil sample, but not oxalic acid, etc., should be halved. Then the soil %N should be initially computed as if no change were made and this answer doubled for the true value. Soils with very high organic-N content, such as peats, may be run by making further sample size reductions. These samples, however, should be initially ground with mortar and pestle to more completely expose the higher organic fraction to the dye.

RESULTS AND DISCUSSIONSoils Used

To test the method on agricultural soils with varying characteristics, a total of 22 samples were obtained from Alabama, California, Idaho, Illinois, Indiana, Montana, Oregon, and Washington. Exchangeable cations, pH, OM (Walkley-Black without external heat),  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , Kjeldahl-N (with salicylic acid for including  $\text{NO}_3\text{-N}$ ), free carbonates, and free iron oxides were determined by conventional methods.

Results of Proposed Method

As shown in Fig. 1, there is good correlation ( $r = 0.93$ )

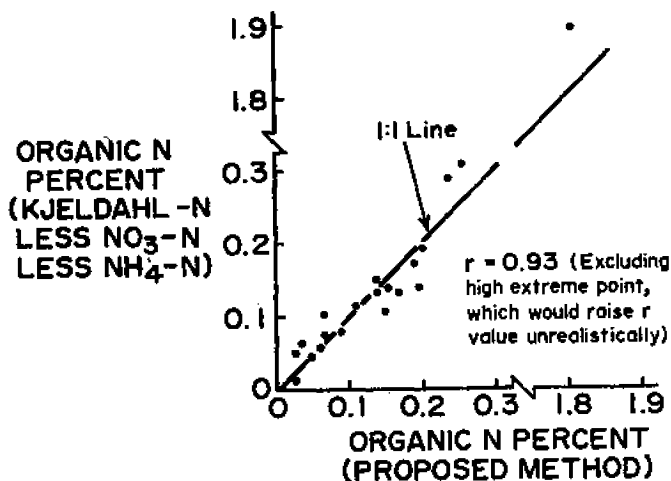


FIG. 1

Comparison of proposed dye method vs traditional organic-N determination

between the proposed method for organic-N and traditional methods (i.e., Kjeldahl N less  $\text{NO}_4\text{-N}$  less  $\text{NO}_3\text{-N}$ ). Soils used in the

comparison ranged from a very sandy soil with a low organic-N content of 0.015% to a peat soil with 1.92%.

The variation of points about the 1:1 line in Fig. 1 may be attributed mainly to the accuracy of the proposed test. From comparing the results between replications of the dye test, variation was small (C.V. = 2.9%), as was the variation when doing the test on different dates which included using different reagent dye batches (C.V. = 3.1%).

It may be noted that the accuracy, on a percent error basis, was poorest from samples low in organic-N. Doubling the soil sample size for the test did not reduce nor change these errors. Also, measurements and calculations were made to determine if the wash water remaining in the soil sample after decanting was contributing appreciably to error. As there was no increase in the  $r$  value as used in Fig. 1 from going through these extra correction steps, they were left out of the proposed procedure.

Organic-N of two soil samples, one from a free-lime horizon, and one with very high iron oxides, could not be determined because of interferences (see "Interfering Ions") and are not included in Fig. 1.

Multiple regression analysis was used to determine which soil characteristics together with organic-N might influence the absorbance readings of the equilibrium dye extracts. Independent variables included soil pH, OM content, and organic-N. Only organic-N was found to be a contributing factor under this method of eliminating variables. As expected, organic matter content it-

self, when treated as a single independent variable, was correlated with the absorbance readings. Presumably this was because the soil's C:N ratios were somewhat constant.

#### Effect of Sieving and Grinding Soil Samples

Better correlation between the dye test and the traditional method mentioned for organic-N was obtained by passing the soil through a 0.5-mm sieve ( $r = 0.93$ ), rather than a 2-mm sieve ( $r = 0.88$ ). Apparently this resulted from increased dye binding when the soil was finely ground. However, further grinding the soil in a mortar and pestle had no effect except for the peat soil. Before grinding the peat soil by mortar and pestle, dye binding was limited to 80% of the binding obtained after the extra grinding — with the extra grinding producing final results differing less than 5% from the traditional method.

#### Effect of Washing, Dye Equilibrium Time, and Dye Stability

Comparisons were made where soils were machine shaken for 5, 15, 30, or 60 minutes during washing, and also during the dye equilibrium period. No differences in dye binding could be found because of shaking times, and therefore the 15-minute time was considered sufficient. Also, the hand shaking alternative proposal (see PROPOSED METHOD) was compared to the machine shaking process and results were identical.

Several colorimetric comparisons were made between newly mixed reagent dye and older batches that had stood for up to several months in a clear pyrex bottle. The comparisons indicated that there was no change in dye with aging. Also, comparisons

were made of soil equilibrium dye extracts that had aged for up to a week and no differences were found. However, calibration curves were checked several times each day for colorimeter drift.

### Interfering Ions

Calcium and heavy metals were known to form chelation products with the dye<sup>4</sup>, resulting in a loss of color intensity. To test which common soil ions could produce interferences, 0.05 g of all possible combinations of carbonates, sulfates, and chlorides of calcium, magnesium, potassium, and sodium were added to a neutral silt loam soil. Only the  $\text{CaCO}_3$ ,  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ , and  $\text{MgCO}_3$  caused measurable dye color loss. (Chlorides have previously been found to intensify the dye<sup>4</sup> and probably there was an off-setting effect with this ion.) When  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  were added to soils, neither caused a change in dye readings.

Of the soils analyzed, high calcium soils tended to give the most difficulty. The addition of the oxalic acid stabilized the absorbance readings of these soils and did not affect the absorbance readings of neutral or acid soils. However, a soil from a lime layer with 15% free  $\text{CaCO}_3$  could not be thus stabilized. Associated with the free  $\text{CaCO}_3$  in this soil, the pH of the centrifuged dye solution was 4.1, whereas all other soils produced a dye solution near 3.0. Thus, this measurement helped to distinguish soils that cannot be analyzed by this method.

Soils with up to 5% free iron oxides were analyzed quite accurately and the oxalic acid helped to reduce the extra adsorption of dye by this material. However, the N content of one



soil with 6.2% free iron oxides and only 0.04% organic-N could not be determined because of the overwhelming amount of iron oxide as compared with the organic-N content.

Thus, except for high calcium soils or those with large amounts of free iron oxides, the Udy method provided a convenient and fairly accurate estimate of organic-N. Its further adaptation should rely on comparison of results with traditional organic-N determinations for particular soils in question. The number of samples capable of being run per day will depend mainly on centrifuge capacity, then next on "automaticness" of pipettes, diluters, and colorimeter.

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