Today farmers must produce high yields to survive in the face of the growing costs of production. High amounts of available nitrogen are essential for high yields although excessive nitrogen reduces the processing quality of the roots. Plant tissue analyses have proven valuable in determining whether proper amounts of nitrogen have been used. In addition, soil analysis for nitrate nitrogen is helpful in estimating the amounts of nitrogen fertilizer to apply. Nitrate nitrogen is not the only source of nitrogen available to plants, but it cannot be ignored when present in large amounts. For example, soils in the sugarbeet growing area of Eastern Oregon and Southwestern Idaho have been found to contain from 25 to 325 pounds of nitrate nitrogen per acre in the top 2 feet of soil prior to fertilization for a sugarbeet crop.

The analysis of large numbers of plant or soil samples is hindered by the lack of a method which will quantitatively measure nitrate nitrogen in a relatively fast and easy manner. Hartley and Asai (1)3 describe a method for water analysis which is simple to use, direct and rapid. They state that “The precision of the method expresses as relative standard deviation averages less than 1% in the nitrate range of 5-20 P.P.M. nitrate nitrogen (original sample concentration). Because of the extreme rapidity in carrying out a determination and its conformity to Beer’s law, the 2, 6-dimethyl phenol procedure should be very useful for determination of nitrate in samples containing nitrate nitrogen in the parts per million ranges.” Their method as adapted for plant and soil analysis is given below.

**Procedure for Plant Tissue**

Weigh out 0.5 or 1.0 g. of dried ground plant material, depending on nitrate nitrogen content. If nitrate nitrogen is above 15,000 ppm, use 0.5 g. or change dilution of extract from that recommended below. Add 100 ml of 2 percent acetic (V/V) to each sample and shake for 10 minutes on a platform or wrist action shaker and filter. Two ml of these filtered extracts are diluted to 50 ml with distilled water. In addition to nitrate nitrogen the

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3Numbers in parentheses refer to literature cited.
solutions may now be used to determine phosphorus, potassium and sodium if desired.

Two ml of the diluted extracts are pipeted into test tubes. The test tubes are placed in a container of water at room temperature (18-28°C). Eight ml of 2:1 sulfuric acid-water (V/V) is added to each test tube. The tubes may be removed from the water bath while adding the acid but must be returned to the water bath as soon as possible. When the solutions have cooled, add 2 ml of 2,6-dimethyl phenol in glacial acetic acid, (12.217 g per liter glacial acetic acid). Stopper the tubes, shake each sample separately to mix and place back in water immediately. The percent transmission may be read at 325 Mu 15 minutes after but no longer than 1 hour after mixing with the 2, 6-dimethyl phenol reagent. The solution must now be kept in the water bath until read or the reaction will proceed uncontrolled and results will be erratic and high. A reagent blank is carried through with each set of determinations.

Procedure for Soils

Weigh 5.0 grams of soil, add 50 ml of one normal ammonium acetate (pH 7.0) and shake for 5 minutes. Filter the sample and develop the colors as in the procedure for plant material. Soil extracts usually do not need to be diluted. This extract also may be used to determine available potassium.

Comparative Accuracy and Factors Affecting the Analysis

To determine the comparative accuracy of this method it was compared with a method for plant analysis for nitrate nitrogen similar to that of Ulrich, et al (2) in which phenol disulfonic acid was used. Eight petiole samples ranging from 3000 to 12000 ppm nitrate nitrogen were analyzed by both methods. A simple correlation between the two values gave an r value of 0.99. The average value using phenol disulfonic acid was 7950 ppm and 7446 ppm using 2, 6-dimethyl phenol. A similar comparison between the two methods with 20 soils gave an r value of 0.97. The average value here was 50 ppm with both methods. The exceptionally high r values show that the two methods gave basically the same results.

Hartly and Asai (1) describe interferences from temperature, nitrite nitrogen, chlorides and organic matter. These interferences are discussed below as they affect the analysis of plants and soils.

Temperature affects the reaction by decreasing reaction time as the temperature is increased. This does not affect the determination providing the analytical procedure is followed as outlined. Results will be high and erratic when the temperature of the
solutions are allowed to rise above room temperature after the 2, 6-dimethyl phenol reagent has been added to the reaction. Nitrite nitrogen interferes directly with the reaction (Table 1). Table 1 shows that the extent of the interference depends directly on the amount of nitrite present. Actually nitrite nitrogen could be determined with the same procedure in the absence of nitrate nitrogen. The presence of nitrates can be easily detected because of a yellow color in a lake on the top of the solution that is formed when 2, 6-dimethyl phenol reagent is added to develop the color. The yellow color rapidly turns to orange when the sample is shaken indicating the presence of nitrite nitrogen. The addition of a small crystal of sulfamic acid to the pipeted sample before any other reagents are added will remove nitrates without affecting the nitrate determination. Chlorides in excess of 200 ppm in the diluted sample will cause interference. To obtain this high level plant material must contain over 50% chloride and soil samples must contain over 4000 pounds chloride per acre to a depth of 6 inches of soil. This is true only when samples are diluted as the procedure indicates. Organic matter does not appear to be a problem in the method mainly because the high dilution of plant samples; and neutral normal ammonium acetate does not remove organic matter from the low organic matter soils where this method has been used.

The presence of sucrose was found to interfere with the determination of nitrate nitrogen. Samples from sugarbeet petioles produce a reddish color when analyzed which soil samples and standard solution do not. This red color is very pronounced when samples of fresh beet pulp are analyzed. The cause of the red color was found to be due to fructose which is formed when sucrose present is hydrolyzed in the hot acidic solution to glucose and fructose. This occurs when the sulfuric acid is added to the pipeted sample. The fructose, not glucose, then reacts with the solution to produce the red color. The amount of sucrose or fructose present determines the extent of the interference as seen in Table 2. The greater the concentration in solution the higher the percent recovery of nitrate nitrogen since the two are

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Table 1.—Effect of Nitrite nitrogen on nitrate nitrogen recovery.

<table>
<thead>
<tr>
<th>Nitrite nitrogen (ppm)</th>
<th>Nitrate nitrogen (ppm)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>190</td>
</tr>
<tr>
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<td>1</td>
<td>240</td>
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<tr>
<td>6</td>
<td>1</td>
<td>380</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>435</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>490</td>
</tr>
</tbody>
</table>
additive. Fresh beet pulp is comparatively low in nitrate nitrogen (usually less than 500 ppm) and, therefore, cannot be diluted as much as petiole solutions. As a consequence, the sucrose present gives high values in nitrate nitrogen determination of pulp samples. It is concluded that this method cannot be used for extracts of fresh beet pulp or for any solution which contains more than 0.01 per cent sucrose. Because of the great dilution in the procedure, no interference will occur in a plant sample containing less than 25 percent sucrose. This is higher than the concentration occurring in most plant material. This does not limit the method however, because this method, just as any colorimetric method, is not as accurate as the present transmission of the solution approach 100 percent. In other words, this method as outlined is less accurate when the nitrate nitrogen is less than 1000 ppm. This could easily be corrected by not diluting as much, but this may bring the concentration of sucrose up to a point where it will cause high nitrate values.

The standard curve for nitrate nitrogen using Baush and Lomb Spectronic 20 spectrophotometer at a wave length of 340 millimicrons is given below. Full scale deflection cannot be obtained on this spectrophotometer at 325 Mu as recommended in the original paper. The per cent transmission for 1, 2, 3, 4, 5, 6, 7, and 8 ppm nitrate nitrogen are 80.5, 65.0, 53.0, 41.0, 33.5, 27.0, 22.0 and 17.5 respectively.

### Conclusions

Hartley and Asai (1) concluded that, "Use of 2, 6-Xylenol as a reagent for the spectrophotometric determination of nitrate compares favorable with three colorimetric methods most commonly used for nitrate determinations: the phenoldisulfonic acid, 2, 4-Xylenol, and brucine procedures. This procedure compares favorable with the above mentioned colorimetric methods on the basis of the four requirements: rapidity, specificity, sensitivity, and reproducibility."
The main value of this procedure is the comparative ease of use as a routine method for large numbers of samples which must be analyzed in a short period of time with reasonable accuracy. In actual use the need for special precautions, as used in other methods such as silver sulfate for chlorides, sulfamic acid for nitrites and the use of chelating agents, have not proven necessary. These precautions can easily be incorporated into the procedure if needed.
