APPLICATIONS OF SPECTROPHOTOMETRIC METHODS TO PROBLEMS OF CHEMICAL ANALYSIS IN THE STUDY OF PLANT PHYSIOLOGY

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In the course of a study of the effect of soil and other environmental factors on the development, growth, and composition of cereal grasses, it was found necessary to carry out a large number of analyses on samples of soil and of plant tissues. Standard macro quantitative procedures were used in many instances, but limitations in the size of the samples frequently made it necessary to turn to semi-micro methods. Colorimetric methods using a spectrophotometer were found to be of great value. The speed and accuracy of spectrophotometric methods have led the author to abandon the usual macro procedures in many instances. The purpose of the present note is to call to the attention of plant physiologists the great usefulness of these procedures.

A survey of the literature shows that many colorimetric procedures are available. Reitemeier (14) published a set of methods for the semi-micro-analysis of saline salt solutions. Peech (12) outlined methods for the micro-determination of exchangeable bases in soils. Wall (16) developed micromethods for the determination of inorganic constituents of plants. Lindner (8) has also outlined colorimetric methods for the rapid analysis of the common inorganic constituents of plant tissue. Parks, Hood, Hurwitz and Ellis (11) set up a systematic procedure for quantitative microdetermination of twelve elements in plant tissue. Wolf (18) described several rapid photometric methods for use on plant tissues. Numerous other methods are also available for the analysis of plant material.

Many colorimetric methods have been tried in the laboratory over a period of several years for the analysis of soils and plant materials. The present paper does not intend to describe the details of the methods used, but merely to suggest a few of the most generally applicable types of analyses possible through the use of a spectrophotometer. All the methods mentioned have been thoroughly tested by experience and they are especially valuable because of their precision and the comparative ease of execution. A Coleman Model 11 Universal Spectrophotometer was used for all determinations. Photometric calibration curves were prepared from standards, but it was found advisable to run a series of standards through the colorimetric procedure with each day's run.

COLORIMETRIC METHODS OF SOIL ANALYSIS

Phosphorus fractions.—Bray and Dickman (2) described procedures for the quantitative determination of four fractions of phosphorus adsorbed by the colloidal material in the soil. The phosphorus in the various types of extracts was determined colorimetrically by the method of Dickman and Bray (4) using the Kurtz (7) modification to suppress interference by the fluoride ions. A wave length of 7750 A was used to-

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gether with a PC-5 filter. Standards were prepared containing 0.005 to 0.065 mgm. of phosphorus per 50 ml.

Potassium.—Exchangeable potassium was determined in the ammonium acetate leachate obtained during the base-exchange procedure as described by Noggle and Wynd (10). After evaporation of the leachate, it was ignited and taken up with nitric acid, and the potassium determined by the method of Wander (17). A wave length of 4250 A was used with a PC-4 filter. The standards contained 1 to 7 mgm. of potassium per 100 ml.

Sodium. — Exchangeable sodium in the ammonium acetate leachate, obtained by the conventional base-exchange procedure (10), or water-soluble sodium, was determined colorimetrically as described by Reitemeier (14). A wave length of 4200 A was used with a PC-4 filter. The sodium standards contained 0.025 to 0.25 mgm. of sodium.

COLORIMETRIC METHODS FOR THE ANALYSIS OF PLANT ASH

The inorganic constituents in plant tissues were obtained in solution by wet ashing with perchloric and nitric acid; 0.5 to 1.0 gm. of dried plant material was treated with 10 ml. of water, 10 ml. of concentrated nitric acid and 10 ml. of 72 percent perchloric acid. The beaker, containing the plant material, was covered with a watch glass and the contents boiled gently on a hot plate until the organic matter was destroyed. After cooling, the watch glass and sides of the beaker were washed down with distilled water. The beaker without the watch glass was then returned to the hot plate and the contents evaporatad to dryness. The residue was taken up with 5 ml. of 1:4 HCl and 25 ml. of distilled water. The solution was then filtered to remove the silica which was dehydrated by the perchloric acid. The filtrate was made up to 100 ml. volume and aliquots used for subsequent analysis.

Magnesium.—The method of Reitemeier (14) was used to determine magnesium. An aliquot of the ashed solution was transferred to a 15 ml. centrifuge tube and the calcium precipitated as the oxalate. The filtrate and washings from the calcium oxalate precipitate were made up to 25 ml. volume and an aliquot taken for the magnesium determination. The magnesium was precipitated as magnesium ammonium phosphate hexahydrate, and the amount estimated from the phosphorus content. Solutions containing 0.01 to 0.08 mgm. of magnesium were used as standards. A wave length of 5400 A was used with a PC-4 filter.

Potassium. — The cobaltinitrite procedure, using the nitroso R-salt, as outlined by Peech (12) was used. A wave length of 5500 A in connection with a PC-4 filter was used. Standards contained 0.1 to 1.8 mgm. of potassium.

Phosphorus. — The colorimetric phosphate method of Dickman and Bray (4) was used. A wave length of 7750 A and a PC-5 filter were used. The standards contained 0.005 to 0.065 mgm. of phosphorus.

Manganese.—The periodate method as described by Peech (12) was found to be very satisfactory. A wave length of 5350 A and a PC-4 filter were used. The standards contained 0.005 to 0.20 mgm. of manganese.

Iron.—The method of Hummel and Willard (5) as modified by the AOAC (1) was used. A wave length of 5100 A and a PC-4 filter were used. Solutions containing 0.01 to

0.09 mgm. of iron were used as standards.

COLORIMETRIC METHODS FOR NITROGEN

Boiling water extracts were made of the dried plant material. Nitrate, ammonia, alpha-amino, and amide nitrogen were determined in the extract. The scheme of analysis as outlined by Schlenker (15) was used. All fractions, except nitrate nitrogen, were determined by Nesslerization of ammonia after alkaline aspiration.

Ammonia nitrogen. — The ammonia, after being aspirated from an alkaline solution into 0.1 N HCl, was determined colorimetrically by Nessler's reagent. The Nessler reagent was prepared according to Koch and McMeekin (6). A wave length of 5000 A was used with a PC-4 filter. The standards contained 0.1 to 1.0 mgm. of ammonia nitrogen.

Nitrate nitrogen.—The nitrate nitrogen was determined colorimetrically by the phenol-disulphonic acid method as outlined by Schlenker (15). A wave length of 4100 A with a PC-4 filter was used. 0.01 to 4.0 mgm. of nitrate nitrogen were used as standards.

METHODS FOR ASCORBIC ACID AND CHLOROPHYLL

Ascorbic acid.—The ascorbic acid was extracted by disintegrating the plant material in a Waring Blendor. The extracting medium was 0.5 percent oxalic acid as suggested by Ponting (13). The ascorbic acid was determined by the method of Morell (9). A wave length of 5200 A was used with a PC-4 filter. Standards containing 1 to 14 micrograms of ascorbic acid per ml. were used.

Chlorophyll.—The chlorophyll was determined by the method of Comar, Benne, and Buteyn (13). The chlorophyll was extracted by disintegrating the tissue in 85 percent acetone with a Waring Blendor. A wave length of 6600 A was used with a PC-5 filter.

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