A COMPARISON OF FOLIAR AND SOIL TESTS TO DETERMINE MINERAL DEFICIENCIES

by

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INTRODUCTION

The determination of mineral constituents of plants is of importance in connection with many fertilizer investigations. Up to the present, interest has been mainly centered in the amounts of the different elements present in plants, these being an indication of their relative availability in the soil.

Foliar analyses have long been used to gain insight into the problems of plant nutrition. However, until recently, the number of samples that could be analyzed was limited by the lengthy procedures necessary for the chemical determinations. Colorimetric photometry and emission spectroscopy have simplified analytical procedures. A new device, the atomic absorption spectrophotometer, has been introduced recently which is still more efficient and might replace almost all other methods in time.

As a result of the broader view now taken with respect to the problems of soil fertility, it is recognized that the conditions required for maximum growth must be sought in the areas of plant physiology as well as from soil science. For satisfactory growth a particular soil must satisfy certain conditions with respect to temperature, and nutrient, water and air supply to roots. The task of investigation is to discover the best means of bringing the factors that govern these conditions under control. For this purpose the traditional chemical methods, both of plant and of soil analyses,

have definite limitations; some have been abandoned, but others are still in use (83).

In recent years interest has been renewed in the possibilities of the chemical analyses of the plant as a means of studying the nutrient relationships between the crop and the soil with respect to (1) the physiological requirement of a particular species, and (2) the rate of supply of nutrients (36).

The specific objectives of this investigation are (1) to investigate the effect of the addition of certain nutrient elements to the soil upon leaf concentrations of these and other elements; (2) to establish, if possible, critical lower limits of all nutrients, especially nitrogen, phosphorus and potassium in leaves of plants; and (3) to ascertain whether or not soil deficiencies of various nutrient elements could be detected by leaf analysis.

As plant growth is a function of both the quantity of nutrients, sometimes designated as "intensity," and the "nutrient balance," Shear and others maintain that the purpose of leaf analysis is to find out both the extent of nutrient unbalance in the plant and whether excesses or deficiencies exist (78).

HISTORY

It is only within the last few years that much attention has been focused on foliar symptoms as a means of determining nutrient deficiencies. The method is not new. As

early as 1804, De Saussure analyzed the ash of plants and observed that its composition varied with the soil, the part of the plant analyzed, and the age of the plant (94). In 1844, the French scientist Gris called attention to chlorosis due to iron deficiency. Somewhat later (1849-51) Salm-Horstman described plant growth characteristics resulting from each of the elements that were known to be essential at that time (58). In 1869, Hellriegel, according to Hall (30), discussed the relative varieties of the proportion of potash in the ash of barley straw and the soil in which it was grown. Henrich, Atterberg and Godlewski each reported work on plant analysis before 1900 (30). Hall's report of work done at Rothamsted was published in 1905. This was undertaken in an effort to ascertain plant nutritional needs more accurately than can be done with soil tests. In 1930, two Frenchmen, H. Lagatu and L. Maume (62), emphasized the value of deficiency symptoms in studying nutrient requirements of potatoes. Two of the earlier critics of foliar symptoms were Murneek and Gildehaus, who published an article in Science in 1931 (62b). Hall (30) commented that the idea of determining soil deficiencies by means of the analyses involves taking a plant from the soil under study and determining the proportion of phosphoric acid, potash, and other constituents in its ash. A deficiency or excess of a particular constituent is indicated by the variation of the amount present from the normal.

Hall's work led Salter and Ames (72) to conclude that the uptake of any nutrient was influenced by so many factors as to preclude the hope of using plant composition as a guide to soil requirements unless the influence of these factors was controlled or measured. These workers stated that the tendency for a nutrient element to vary directly in the plant with the supply in the soil held better for nitrogen and potassium than for phosphorus. Chubb and Atkinson (19) concluded that there was no simple and direct relationship between the compositions of the leaves of the plants which they analyzed and the additions of the elements of the soil.

FOLIAR DIAGNOSIS

Foliar diagnosis is characterized as the chemical composition of a leaf with respect to the dominant nutritive mineral entities at the instant of sampling, taken from a predetermined and suitable position on the stalk. The foliar diagnosis for any given season (year) then will consist of a sequence of chemical states (composition) as determined under (a) on different dates, that is, at different periods during the growth season(83).

While describing the importance of foliar diagnosis, Lagatu and Maume state that of the numerous methods employed to control the nutrition of plants under the condition of practical agriculture only the method of foliar diagnosis has any serious claim to be based on physiological facts

established by consistent experimental results obtained over a long period of years (46).

The foliar diagnosis of a particular plant species at any given instant of time consists of the mineral composition of a leaf from a definite rank (metabiotic age) on the stem. The chemical state with respect to a leaf of the same rank of a particular species as determined at different dates characterizes the foliar diagnosis of that particular plant species (and variety) for the particular growth season examined (83).

The application of a complete fertilizer compared with one containing nitrogen only has, accordingly, increased the intensity of nutrition, and has acted as a "brake" on the absorption of nitrogen, indicated as being too high in nitrogen. The effect of these changes on the quantity and quality of the nutrition of N-P-K is favorable to the yield (84).

There are many plants which are good indicators for most of the nutrients. Wallace (98) stated that among fruit crops, the apple is a suitable indicator plant for nitrogen, calcium, magnesium, potassium, iron, manganese, boron, copper, and zinc. Due to the deficiency of these nutrients, apples show the following various symptoms:

Nitrogen deficiencies. Shoots short and thin; foliage sparse, leaves small, pale green and develop bright orange, red or purple tints; defoliation early, fruit buds and

blossoms sparse, fruits small and highly colored.

Phosphorus deficiencies. Shoots short and thin; foliage sparse; leaves small, dull green, and develop purple or bronzed tints; defoliation early; fruit buds and blossoms sparse; fruits small, color variable.

Calcium deficiencies. Tips of shoots die back; leaf margins ragged, scorched and turned toward upper surfaces; scorching develops progressively from young to old leaves.

Magnesium deficiencies. Shoot growth may vary from normal to practically nil; leaves severe intervenal necrosis, either centrally near midrib or beginning near margins, towards end of season; may also show intervenal chlorosis and or purpling; defoliation of terminal shoots, beginning at bases, severe, and trees may be practically defoliated by August; fruit may fail to ripen when defoliation severe.

Potassium deficiencies. Shoots thin or grow stunted and the branches die back; leaves bluish-green and may be slightly chlorotic near margins; leaf margins scorched; either grey or brown color; fruits small, immature appearance but color variable.

Iron deficiencies. Leaves at tips of terminal shoots chlorotic; small veins may show in detail or leaves may be entirely bleached and margins show some brown patches; chlorosis decreases down shoots; branches may die back; fruits pale ground color and highly flushed.

Manganese deficiencies. Leaves intervenal chlorosis (generally V-shaped pattern) beginning near margins and

progressing towards midrib; small veins not visible in chlorotic areas; chlorosis may be general over tree and young leaves on shoots may be green.

Boron deficiencies. Shoots show some defoliation and terminal leaves resemble rosette condition; bark may be rough and split; fruit malformed, skin cracks and pits, flesh may show brown spotting or browning in core region.

Copper deficiencies. Young shoots may make normal growth early in season but by early summer young tip leaves show distortion and browning and are shed; this defoliation proceeds progressively down the shoots, accompanied by shoot die-back; the barks of the trees may become rough and may separate from the wood; in later stages large shoots die, sucker growths are developed from bases of stems and these also subsequently die back from tips, fruiting becomes negligible.

Zinc deficiencies. Shoot growth dwarfed and internodes become progressively shorter until leaves only carried as bushy rosettes or terminal growths; leaves are small and narrow and may show some intervenal chlorosis; fruits severely dwarfed.

SAMPLING

Chubb and Atkinson (19) cited a few conclusions by Thomas and Mack of Pennsylvania after an extensive experimental work on the foliar diagnosis technique proposed by Lagatu and Maume:

(a) They indicated that on homogeneous growth media and under the same experimental factors, leaves of the same metabolic age and from the plants of the same kind will give substantially the same chemical analysis (foliar diagnosis).

(b) Leaves from the same kind of plant but grown on different media or subjected to different external factors will have, in general, different foliar diagnosis.

(c) As the foliar diagnoses are subject to variation due to external factors, such as weather, it is impossible to use the figures obtained from any one as absolute values. Such ideal conditions are thus not applicable in field experiments, but control can be sufficiently close to make comparison valid. Many workers also agree about this control.

Tyner (93) proposed critical leaf nutrient concentration for nitrogen, phosphorus and potassium after analyzing the sixth basal leaf of corn plants. Ulrich (94) defined critical nutrient concentration in this way: "In the present paper the critical nutrient concentration of a plant, or part of a plant, is defined as that narrow range of concentrations at which the growth rate or yield first begins to decrease in comparison to plants at a higher nutrient level." In the same article Ulrich advanced a very strong argument in favor of foliar analyses as a diagnostic technique. His argument was that the nutritional status of a plant, or plant part, was an integration of all the factors

that have influenced the uptake of nutrients at the time of sampling. So, when nutrient concentration was less than the established critical level, it was considered that the nutrient was deficient and the reason that the element was deficient may or may not be due to the supply of the element in the soil. A particular plant, time of sampling, climate, management, quantity of elements added and nature of the soil were factors which were believed to influence nutrient uptake.

In sampling leaves of forest trees Mitchell (61) indicated that the period a few weeks before yellowing is best because at that time the maximum amounts of nitrogen, phosphorus, and potassium are present. It is evident that the objectives of foresters and the growers of fruits or field crops are different and hence their procedure will vary.

Thomas (85) pointed out that in fruit and field crops the earlier the leaf samples are taken the better, when only one sampling is to be made. He based this conclusion on these considerations: (1) The earlier the sampling, the better the chance of correcting deficiencies. (2) In the early stages of growth, especially before flowering, the rate of nutrient uptake is greater than when the plant is more mature. (3) As maturity approaches the various nutrients are not always removed from the leaves in proportion to the amounts present. (4) The translocation of nutrients from mature leaves is greatest during the period

of rapid growth, hence the greatest changes in nutrient concentration are occurring at this time.

Shear and his associates (78) stated that when proper consideration is given to the position of the leaves on the shoot and to the sampling time, leaf composition presents a measure of both internal and external environmental factors that influenced nutrient accumulation by the plant. And, because gradients occur in the amounts of each element contained in the leaves that vary (a) during the growth and fruiting season, (b) between shoots producing and those not producing fruit, and (c) in leaves from basal to terminal positions, it is necessary that leaf samples be taken from the same position on nodal shoots and at a time when all shoots are at approximately the same stage of development. The statements of Ulrich (94) also confirm the same arguments.

Thomas (86) believed that plant composition could be used as an index of fertilizer requirements. His article contains an excellent review of the literature to 1945. He believed that greater uniformity of methodology and reporting of results was needed. Thomas also pointed out the difference between earlier and more modern methods. Formerly, plant analyses were carried out on the whole mature plant while at present leaf analyses are based on samples of the functioning assimilating portion of the plant. He further believed that this change is very important in explaining the success of recent workers after repeated failures of

early workers to establish critical levels.

In describing the basis of composition Thomas (82) said that the composition is based on the dry matter of the leaf without taking into consideration the weight of the dry material at each sampling or the number of leaves sampled from each plant. So no physiological significance can be attributed to the foliar diagnosis of any one fertilizer treatment (plot) considered alone. The method is comparative just as the method of analysis of entire plants is comparative. Foliar diagnosis considered independently of all other field data and of all other foliar diagnosis has no physiological significance.

Some workers separate the petioles and analyze the blades only; others remove the midribs also. Some investigators extract the dry leaf tissue with hot water and others use alcohol as an extractant. For determination of the nutrient status of crops and the soils in which they grow it is customary to analyze the entire leaf, after drying at a low temperature (70°C.), for the total quantities of the various nutrients (58).

According to Thomas and Mack (87) the most suitable leaf for sampling was the eldest leaf that remained healthy and functioning during the sampling period. They suggested that the older leaves reflected the stage of internal starvation before younger leaves because nutrients were drawn from the older leaves more rapidly when growth was taking

place. This applies, of course, only to those elements which are translocated. Chubb and Atkinson (19), Ellis, et al (25) and Knauss (44) used the third basal leaf of the corn stalk.

Tissue analyses and tissue testing has increased in recent times. Beers (11) has written on the subject urging farmers to use the method to aid in determining their fertilizer needs. The results are called "quick tissue tests" (16 and 76).

Scarseth (76) said that tissue tests only indicate which element was the first limiting growth factor at the time that the test was made. He suggested that the plant may be unable to use a portion of the nutrients taken up because of a deficiency of one element. This may result in an accumulation of other nutrients, causing them to appear to be present in adequate amounts when a time test is performed. These elements actually might be deficient if the deficiency of the first limiting element were corrected.

Caldwell and McGregor (16) used the Purdue tissue testing kit to reveal a potassium-nitrogen relationship in corn. They found that when leaf potassium was deficient, nitrate nitrogen also was deficient in the plant even when nitrogen had been added to the soil. When leaf potash was sufficient, the leaf nitrate was sufficient whether or not nitrogen had been added to the soil. The soil on which the crop tested was growing had grown a legume for four years

previously. Atkinson, et al (6) found that an increase in nitrate nitrogen was accompanied by a decrease in phosphorus. A decrease in nitrate nitrogen resulted in an increase in phosphorus.

Lundegardh (49 and 50) found an antagonism between potassium and calcium as well as between potassium and magnesium. He suggested that if potassium is present in high concentration, calcium deficiency may occur. If soil potassium is sufficiently low, harmful quantities of leaf calcium may accumulate. Magnesium also may accumulate but not necessarily in harmful quantities if potassium is low. Hoagland and Martin (36) indicated a definite relationship between the uptake of potassium, calcium and magnesium. Plant growth was not limited by specific ratios of these elements.

Hoffer (37) stated that the supply of potassium may be adequate for normal leaf growth if nitrogen is relatively unavailable. As the nitrogen-potassium ratio is widened, metabolism may be affected.

The question arises, why is leaf analysis considered an index of the nutrient status of both the plant and soil? Lundegardh (48) pointed out that the absorbing power of the roots regulates in part the concentration of salts in the leaves and that the nutrient salt transformations taking place in the green assimilating leaves control the growth of the plant, including seed formation. Also, he believes

that leaf analysis not only gives a summation of the extraction of salts from the soil during a period of several weeks, but in addition presents a picture of soil saturation at the time of sampling.

Hopkins (38) described complete soil analysis and proposed that 2 per cent of the nitrogen, 1 per cent of the phosphorus, and 0.25 per cent of the potassium would become available during a growing season under satisfactory conditions of moisture, temperature, soil structure, and so forth.

After the complete soil analysis, Miller (58) emphasized on "partial soil analysis." Instead of entirely decomposing the soil particles, as done in the total analysis method, the soil was extracted with a strong acid. Usually (1.125 sp. gr.) hydrochloric acid of constant boiling concentrated was used and the soil extracted at boiling temperature. This treatment dissolved more of most nutrients than a crop could absorb in the current season, and it was considered that the quantities of nutrients extracted represented the amounts that would become available during a period of years. Extraction with weak acid and with water and soil solution were also proposed.

METHODS FOR ANALYSIS OF THE SOIL

The determination of total nitrogen in the soil. Gedroits (29) stated that nitrogen appears in the soil exclusively in the organic form. The amount of inorganic

nitrogen (ammonia, nitric and nitrous acid) in the soil is as a rule so small that it cannot affect the determination of total nitrogen, being within the limit of the experimental error of the method. For this reason, out of the two known methods of transforming organic to mineral nitrogen, in order to quantitatively determine the latter, viz., Kjeldahl and Jodlbauer methods, the former method is generally used in soil analysis even though it is known not to account for the mineral nitrogen.

Piper (67) stated that nitrogen is determined in soils by one of the many modifications of the Kjeldahl method in which the organic matter is oxidized by sulfuric acid and the nitrogen converted to ammonia. The various modifications differ from the original in the addition of potassium or sodium sulphate to raise the temperature of the digest and in the use of other catalysts in place of mercury. Ashton (1) compared the catalystic efficiency of selenium and copper sulphate in Kjeldahl's method, and found that the whole of the nitrogen is not converted into ammonium sulphate when the contents of the digestion flask have become clear.

Srinivasan (80) obtained higher results by Kjeldahl's method after moistening the soil. He recommended moistening 5 gm. of soil with 20 cc of water, adding 20 cc of concentrated sulphuric acid and leaving the mixture to stand overnight before the digestion.

Walkley (95) determined nitrogen by Kjeldahl's method with and without water in eleven soils of varied origin and wide pH range. He obtained higher results after the addition of water in the case of only two soils, both of which were strongly alkaline. Very fine grinding without the addition of water gave higher results than adding water before digesting the I mm. sample. He also concluded that the lower results were due not to the production of insoluble contents, such as iron and aluminum sulphate, but to the failure of the crumbs in certain heavy alkaline soils to disperse in the non-polar sulphuric acid.

Piper (67) made the point clear that bumping in Kjeldahl's method does not occur during distillation if the solution is transferred from the digestion flask by decantation, thus separating most of the sand. The addition of a small piece of zinc to the distillation flask promotes smooth boiling and the slow evolution of hydrogen minimizes the danger of the distillate nicking back. Some analysts use copper flasks for the distillation, but borosilicate glass is to be preferred since the contents can be seen.

Walkley (95) found that very fine grinding in a ball-mill ensured complete digestion in similar types of soils. It is not necessary to use Bal's modification to obtain maximum values for nitrogen in all soils. Its general adoption is, however, recommended since it does not add materially to the time required for the determination and

it ensures complete digestion of the organic nitrogen in all soils.

Bal (7) has shown that some heavy clay soils give erroneously low values for nitrogen unless the soil is allowed to stand with water before digestion. If this preliminary soaking with water is omitted, nitrogen within the clay aggregates is only partly attacked. Ashton (1) pointed out that the usual methods do not include the whole of the nitrogen of the soil for most of the nitrate nitrogen is lost during the digestion. This loss may be disregarded for most soils since the amount of nitrate nitrogen is negligible in comparison with the organic nitrogen. If, however, it becomes necessary to include it, Ulsch's method should be used. In this method the nitrate is reduced, by finely powdered iron and dilute sulphuric acid, before commencing the digestion. Piper (67) pointed out that the ammonia, which is distilled off from the digest, is usually absorbed in standard acid. Markley (52), while mentioning Winkler's modification, said that this ammonia is absorbed in an unstandardized solution of boric acid. Since boric acid is a very weak acid, the absorbed ammonia can be determined by titration with standard hydrochloric acid, if a suitable indicator (brom phenol blue) is used. This gives a direct determination, in place of the usual indirect determination by difference, and only one standard solution is required.

Determination of calcium. An excellent method for the determination of total calcium is the "ammonium oxalate method," in its two equally accurate modifications, (1) the gravimetric and the (2) volumetric determination, provided iron and aluminum have been quantitatively removed without carrying away a part of the calcium (29). In the volumetric method titration is done with potassium permangemate, but a solution of ceric sulfate can also be used for titration (102). (3) Method of J. Labord (45) as modified by H. D. Chapman. The principle of this method is, to the solution being examined free oxalic acid is added (and not ammonium oxalate); the solution is then brought to pH 4 with ammonia on heating, when aluminum, iron and manganese remain in solution as oxalates (17). (4) The method of Labord-Barlet (27) was described by Chapman in 1928. There is another method, that is, (5) alkalimetric determination of calcium (29).

Determination of magnesium. Magnesium is determined by precipitating it as magnesium ammonium phosphate, which is either ignited and weighed as magnesium pyrophosphate or dissolved in standard acid and titrated with standard alkali as in Handy's method (32). In soil work, magnesium is determined in the filtrate from the calcium determination. Before precipitating the magnesium it is necessary to remove ammonium salts. This can be done either by acidifying the solution, evaporating to dryness and igniting (104), or by treatment with nitric acid (35).

There are many conflicting statements about the precipitation of magnesium. This problem was investigated by A. W. Epperson, who found that double precipitation is necessary to ensure the quantitative precipitation of magnesium ammonium phosphate. He also demonstrated that errors due to (1) a very large excess of the precipitant, (2) the presence of potassium chloride and (3) the addition of the precipitant to an ammoniacal solution were not entirely remedied by precipitation. These conditions can be avoided, except that due to excess of potassium chloride. In that case a third precipitation usually gave a correct result (26). Burd and Martin (13) pointed out that ignition can be avoided by dissolving the precipitate, after the removal of free ammonia, in standard acid and titrating the excess of acid with standard alkali. This method is preferable when the quantity of the precipitate is small.

Exchangeable magnesium can also be determined by means of the flame photometer (101).

There are certain other methods of magnesium determination, i.e. (1) gravimetric method of determination of magnesium, modified by precipitation by means of sodium phosphate (77 and 90). (2) A volumetric determination of magnesium (8 and 12). (3) Determination of magnesium with 8-hydroxyquinoline. Hough and Ficklen applied the reaction between magnesium ions and 8-hydroxyquinoline, which proceeds quantitatively in alkaline medium with the formation of a light green precipitate to develop a gravimetric, or volumetric,

and a colorimetric method of determining magnesium (40). (4) The determination of calcium and magnesium by titration in the same solution. Fox considers this method particularly suitable for soil and ash analysis (28).

Determination of potassium. Potassium is determined by precipitating it as potassium chloroplatinate, potassium perchlorate or potassium sodium cobaltinitrite (104).

Various methods:

a. The chloroplatinate method (4).
b. The perchlorate method (21).
c. The gravimetric cobaltinitrite method (31).
d. Lewis and Marmoy's method (47).
e. Piper's method (68).
f. Wilcox's method (100).

Determination of sodium. Gedroits (29) explained the determination of sodium by difference: that the content of Na_2O in the sample of the soil taken for the determination of alkali metals is calculated from the difference between the weight of NaCl + Kcl and that of $K_2 Cl_6$ (if only an aliquot of the solution of alkali chlorides has been taken to determine the content of potassium, the weight of the potassium chloroplatinate obtained must first be recalculated on the total volume of the solution) as indicated on the next page.

The weight of potassium chloroplatinate is multiplied by 0.3056, and hence the corresponding weight of KCl is obtained. The weight of KCl is subtracted from the weight of NaCl + KCl; the weight of NaCl thus obtained is multiplied by 0.5303 (or by 0.3934), giving the content of Na_2O (or Na) in the sample taken.

There are very many other methods for the direct determination of sodium, viz.:

a. The method of H. Pellet (64).

b. The method of D. U. Hill (34).

c. The method of G. Smith (79).

d. Gravimetric zinc uranyl acetate method (9).

e. Volumetric zinc uranyl acetate method according to Dobbins and Byrd (23).

f. Gravimetric determination as magnesium uranyl acetate according to Corley and Foulk (15).

g. Volumetric determination of sodium as magnesium uranyl acetate according to Corley (14).

h. Gravimetric and volumetric magnesium uranyl acetate method according to E. Kathane (42).

Determination of phosphorus. The availability of soil phosphorus to plants varies greatly depending on the reaction, the mineralogical composition, the type of colloids present, and the content of organic matter of soil. In many instances, phosphorus occurs in the soil in the form of difficult compounds which are not readily soluble, such as iron and aluminum phosphate, tri-calcium phosphate, and organic phosphorus (101 and 58).

A content less than 10 pounds of phosphorus, or less than 25 pounds of P_2O_5 per acre indicates acute deficiency of phosphorus for most tree species. A content exceeding 100 pounds of P_2O_5 per acre is satisfactory for exacting hardwoods and conifers. Analysis showing a plentiful supply of available phosphorus need not be questioned especially considering the low phosphate requirements of woody plants in comparison with grain crops. Tests showing a critical deficiency of phosphorus should be regarded with caution and verified by foliar analysis (101).

There are numerous analytical procedures for determining the content of soil phosphorus but preference is given to Truog's method, which has been used for many years in analyses of nursery and woodland soils (92). Phosphoric acid is generally determined in soils either by precipitation as ammonium phosphomolybdate or by Deniges' colorimetric method. Phosphomolybdate precipitate is dissolved in an excess of standard sodium hydroxide solution and the excess of alkali is titrated with standard acid in the presence of phenolpthalein (65 and 66). There are a few other methods for determination of phosphorus, viz.: Woy's method (103), Pemberton's method (5), Truog and Meyer's method (91), Chapman's modification of Truog and Meyer's method (18), Warren and Pugh's method (99), and Molybdenum blue reagent method (105).

Determination of sulfuric acid. Sulfuric acid is determined as barium sulfate. The acid solution is brought

to the boil and a boiling solution of 10 per cent barium chloride added; the boiling is continued for a few more minutes with stirring, and the beaker, covered with a watch glass, is left on a boiling water bath for at least four hours.

The supernatant liquid is tested for complete precipitation, and the solution filtered through a fine ashless filter paper (No. 589_3), the precipitate washed with boiling water, and acidulated with hydrochloric acid, until the washings give a negative reaction for barium; the precipitate together with the filter paper is placed in a weighed platinum crucible, dried, the paper ashed over a weak flame and ignited. The weight of the barium sulfate obtained multiplied by 0.3429 (or 0.4114) will give the amount of sulfuric acid as So_3 (or as So_4) in grams, contained in the soil sample taken, that is, in 2 or 2.5 gms. soil (29).

Determination of boron. In the determination of boron it is necessary to use boron-free glassware. A 20 gr. sample of air dry 20-mesh sieve soil is placed in a 300 cc beaker with 40 ml of water. This mixture is brought to a boil and allowed to simmer for five minutes, after which 0.02 gm of $CrCl_2 \cdot 2H_2$) is added for clarification. The solution is cooled and a 1 ml aliquot is placed in a 250 ml beaker. Four ml of curcumin-oxalic acid solution are added and mixed thoroughly by rotating the beaker. (The rest of the procedure is the same as mentioned for the leaf analysis on page 29) (22).

Determination of iron. According to the statement of Gedroits (29) the methods for determining iron may be classified under two main headings: (1) the iron is determined as ferrous oxide; since soil extracts contain ferric iron, it must be reduced to the ferrous form; (2) iron is determined as ferric iron, so that no preliminary reduction is necessary.

There are a number of methods by which iron can be determined, such as determination of iron by titration of ferrous oxide with permangamate in sulfuric acid solution, titration of ferrous iron in hydrochloric acid solution by permangamate (88), determination of ferrous iron by titration with standard cerium sulfate (102), volumetric determination of ferrous iron by potassium iodate (33), iodometric determination of iron (81), determination of iron by titrating ferric iron against stannous chloride in the presence of methylene blue (71), determination of ferric iron by stannous chloride titration (88, p. 179), and determination of ferric iron by titration against titanous chloride (90).

The most convenient method in practice of all those described is considered to be the iodometric method (29). Swift (81) said that this method is applicable to both hydrochloric and sulfuric acid extracts, and to aqueous soil extracts, and is based on the reaction

$$Fe\,Cl_3 + K\,I = Fe\,Cl_2 + K\,Cl + I$$

When this method is used excess potassium iodide is added to the hydrochloric acid solution in a ground glass-stoppered flask and the flask left stoppered for five minutes. The liberated iodine is then titrated with standard 0.1 N sodium thiosulfate until the color is fully discharged. Excess thiosulfate is added, which is backtitrated with 0.1 N iodine, with starch as indicator, until blue coloration appears (81).

Determination of aluminum. It can be determined both by gravimetric and chlorimetric methods. It can also be determined by difference as oxide, by subtracting phosphoric acid and ferric oxide from the sum of sesquioxides and phosphoric acid. This method is, of course, very inaccurate, yet it is important (29). Direct methods of determination of aluminum are (1) separation of aluminum from iron and differentiation with the aid of an acetyl chloride-acetone mixture. Minning (59) proposed a method for the separation and determination of aluminum, based on the fact that a mixture of acetyl chloride and acetone quantitatively precipitates the hydrated aluminum chloride $AlCl_3\ 6H_2O$ (59 and 60). (2) Separation of aluminum from iron with the aid of ether; this method proposed by Palkin (63).

Determination of manganese. Manganese is determined by the following methods:

a. Gravimetric determination of manganese by oxidation with bromine (89).

b. Gravimetric determination of manganese according to J. Majdel. This method is a modification of Knorre's method for the separation and gravimetric determination of manganese in ores and alloys. Of all the elements, only Pb, Ba, Sr, Sn, Sb and Ti must be removed prior to the determination. Hydrochloric and nitric acids, and chlorides and nitrates interfere, and must be removed by evaporation with sulfuric acid (51).

c. Colorimetric determination of manganese according to B. V. Horvath (39).

METHODS FOR ANALYSIS OF THE PLANT PARTS (TISSUE/LEAF)

Plant analysis can be a successful means of detecting the development of nutritional disorders that a soil test might not predict. The availability of some major mineral elements, essential for plant growth, viz. phosphorus, potassium, calcium and magnesium, and particularly of the micronutrients, i.e. boron, chlorine, copper, iron, manganese, molybdenum and zinc, are not easily measured by means of a soil test. The effect of soil fertility level and fertilizer treatment on the uptake of these elements by plants can be determined by a plant analysis.

Plant analysis is not a substitute for a soil test. It is just another tool to help the farmer produce optimum yields of high quality. A soil test and a plant analysis

can be used effectively in conjunction with each other. Often a recommendation given as a result of a plant analysis calls for a soil test and following its recommendations.

Plant analysis work is carried out efficiently by an "emission spectrograph" and by an "atomic absorption spectrophotometer." Plant samples are analyzed for 12 elements, i.e. phosphorus (P), potassium (K), sodium (Na), calcium Ca), magnesium (Mg), manganese (Mn), iron (Fe), boron (B), copper (Cu), molybdenum (Mo), zinc (Zn), and aluminum (Al).

Determination of nitrogen. Nitrogen is determined by means of the Kjeldahl method. Wilde and Voigt (101) state the method as follows: A 0.5 gm sample of plant tissue is weighed and wrapped in a 9 cm filter paper. The wrapped sample is placed in an 800 ml Kjeldahl flask and digestion and distillation are carried out as in the determination of total nitrogen in soils. Duplicate samples are run, and two blank determinations are made in each series. The content of total nitrogen is calculated from the number of ml of acid consumed in the titration and is reported as a percent of the oven-dry plant tissue.

Determination of calcium and magnesium. For the determination of calcium and magnesium in plant tissue, dry washing is the most rapid and suitable procedure. A 10 ml aliquot of the ash solution is placed in a 100 ml beaker and evaporated to dryness on the hot plate at 80° C. The salts are taken up in 50 ml of 0.4 N HCl and the solution is

filtered. The contents of calcium and magnesium are determined by means of a spectrophotometer and standard curves (101).

Determination of potassium. It is desirable to use wet ashing of plant tissues for the determination of potassium since some potassium is carried out on a 10 ml aliquot of the ash solution which is placed in a 100 ml beaker and evaporated to dryness on a hot plate at 80° C. The salts are taken up in 50 ml of extracting solution which is 2 N with respect to ammonium acetate and 0.2 N with respect to magnesium acetate. The solution is filtered and the potassium content is determined by means of the Perkin-Elmer flame photometer. The values for potassium are obtained from a standard curve. If the Beckman spectrophotometer is used, the salts are taken up in 0.4 N HCl and the solution is filtered. The potassium content of the solution is determined by means of a spectrophotometer and standard curves (101).

Determination of sodium. Sodium is most conveniently determined in a phosphate free solution of the plant ash by precipitation as sodium uranyl magnesium acetate. The weight of precipitate obtained by this method is multiplied by 0.01500 to obtain the amount of sodium (Na) or by 0.02022 to obtain sodium oxide (Na_2O) in the aliquot taken (70).

Determination of phosphorus in plant tissue. Wilde and Voigt discussed a procedure which is a modification of the method described by Barton in 1948 (10). An aliquot

which contains 1 to 15 ppm of phosphorus is taken from the solution obtained from ashing the plant material and placing it in a 50 ml volumetric flask. Ten ml of nitric acid-molybdate-vanadate mixture are added and the solution diluted to volume. The transmission of color is determined after 10 minutes by means of a colorimeter with a 440 mu filter. The content of phosphorus is determined from a standard curve. The amount of phosphorus in the sample is calculated from the size of the original tissue sample used in ashing and the dilution made in the determination; it is reported as a percent of the oven-dry weight. This method gives a very stable color whose transmission can be determined even two weeks after development (101).

Determination of sulfur. Sulfur cannot be determined in the ash obtained by the ordinary methods of ashing. During the ignition of plant materials organic sulfur is oxidized and passes off with the other products of combustion. If these gases are passed through strong oxidizing agents, the oxides of sulfur are absorbed and converted to sulphates, which can be precipitated as barium sulphate. Such methods give reliable results but are not always convenient (67). Marston (54) found that, of several methods investigated, determination by combustion with oxygen in a steel bomb is most convenient and gives the most consistent values for sulfur. The method determines both inorganic and organic sulfur. Complete combustion of the organic matter

is obtained. The silica and basic constituents become fused into clear glass beads, a large proportion of the nitrogen in the sample is burned to nitric acid, and all of the sulfur is converted to sulphate. Volatile compounds of sulfur are not formed. After combustion the contents of the bomb are dissolved in hydrochloric acid, silica separated and sulphate precipitated as barium sulphate in very dilute acid solution. Throughout the determination electric heaters should be used since sulfur may be derived from the coal gas, if this is used as a source of heat.

Determination of boron. The amounts of boron occurring in plant materials are very variable and values range from 2 - 100 mg. per Kg. of dry matter for normal plants. The usual chemical methods for the determination of boron are based either on the titration of boric acid, or the development of a blue color with quinalizarin in concentrated sulfuric acid (67). The following two methods have been used successfully for the determination of boron in fruit and leaves: (1) The determination of boron by Dodd's Method (24), and (2) the determination of boron by Maunsell's Quinalization Method (56).

Dible, Truog and Berger (22) used photoelectric colorimeter and the content of available boron was obtained from a standard curve prepared by analysis of boron solution.

Determination of cobalt. On account of its importance in the nutrition of ruminants it is often necessary to determine cobalt in plant materials. The amounts present are

usually extremely small and variable. There is some seasonal variation in the amount of cobalt present. It is determined by the following methods:

a. Kidson and Askew's method (43).

b. McNaught's method (57). McNaught found that quantities of cobalt from 0.05 to 20 micrograms can be accurately determined.

c. Marston and Dewey's method (55). They have made an exhaustive study of the conditions governing the extraction of cobalt by dithizone and its subsequent determination as the colored complex with nitrose-R-salt.

Cobalt can also be determined by development of cobalt nitrose-R-salt complex according to Bayliss and Pickering (67 p. 326).

Determination of copper. The Association of Official Agricultural Chemists has used the following method in the determination of copper: "Transfer 20 ml of the ash solution to 125 ml separatory funnel. Add 5 ml of ammonium citrate solution and one drop phenolphthalein. Add 1 : 1 NH_4OH until pink citrate. Add 10 ml dithizone in C Cl_4 and shake for 5 minutes. Draw off C Cl_4 phase into 100 ml beaker. Repeat as many times as necessary using 5 ml quantities of dithizone solution and shaking for 5 minutes each time. The extraction is complete when the aqueous phase remains predominantly orange and C Cl_4 phase remains predominantly green in color. Then add 10 ml C Cl_4, shake for

5 minutes, and combine with the CCl_4 extract. The final 10 ml CCl_4 should be pure green. If not, the extraction was incomplete and must be repeated. Add 2 ml of 60 percent perchloric acid to the combined CCl_4 extracts, cover beaker with a pyrex watch glass, and digest on hot plate until colorless. Remove cover glass and evaporate slowly to dryness. Heat only enough to complete evaporation. Dissolve in one ml 0.2 N citric acid, transfer to a 25 ml volumetric flask, and make to volume with redistilled water."

"Transfer 10 ml from the citric acid solution to a 125 ml separatory funnel. Add 2 ml ammonium citrate solution and one drop phenolphthalein. Add 5 ml Na - diethyldithiocarbamate solution. Add 1 : 1 N H_4OH until pink color develops. Add 1 ml CCl_4 and shake for five minutes. Draw off the CCl_4, centrifuge for 5 minutes, and read immediately in a colorimeter with a 430 mu filter. The parts per million of copper in solution is obtained by referring to a standard curve" (2).

Determination of iron. Iron is determined by the method of Saywell and Cunning (75), which was developed in 1937. They explain the method as follows: "A 2 ml aliquot of the ash solution is placed in a test tube graduated at 10 ml. One ml of 10 per cent hydroxylamine hydrochloride solution and 0.5 ml of ortho-phenanthroline are added. A small square of Congo red indicator paper is placed in the solution and ammonium hydroxide is added until the alkaline

end point is reached. Care should be taken to avoid the formation of a precipitate which will result from the addition of excess of hydroxide. The solution is diluted to volume, mixed thoroughly, and its transmission determined in a colorimeter with a 490 mu filter. A reagent blank is used as the reference liquid. The content of iron is determined by referring to a standard curve prepared by analysis of standard solutions."

Determination of manganese. Jackson (41) has explained the method of determining of manganese as below:

"A 10 ml aliquot of the ash solution is placed in a 150 ml beaker with 10 ml of 85% H_3PO_4 are added to the beaker (1) which is covered with a watch glass and placed on a burner. The contents of the beaker are brought to a boil, allowed to cool, and diluted with 10 ml of distilled water. The beaker is rotated to thoroughly mix the solution and approximately 0.2 gm of sodium paraperiodate is added. The beaker is covered and the solution is heated on the hot plate for about 10 minutes after the appearance of a purple color. Then 75 ml of water are added and the heating continued for 40 minutes or until the purple color no longer increases in intensity. Approximately 0.1 gm of periodate is added near the end of the digestion period. The solution is allowed to cool and is transferred to a 100 ml volumetric flask; less than 5 ml of water free of organic matter are used for washing the remainder of the solution. The flask is stoppered and the solution mixed thoroughly

and allowed to come to room temperature. The solution is diluted to volume with distilled water and an aliquot is used for the determination of percent transmission by means of a photoelectric colorimeter with 520 mu filter. The content of manganese is obtained from the standard curve."

Determination of molybdenum. Molybdenum occurs in aerial portions of plants to the extent of 0.5-5 mg. per Kg., although some fruits and seeds may contain up to 50 mg. per Kg. Few determinations of these very small amounts of molybdenum have been made. At the present time it would appear that Marmoy's method (53) is the most suitable for general use.

Determination of zinc. Zinc commonly occurs in plants to the extent of 5-80 mg. per Kg. of dry matter. Amounts below 20-25 mg. per Kg. are probably most frequent. Walkley (96) has shown that a reaction of pH 9.5-10 is favorable for complete extraction, zinc dithizonate being stable up to pH10.5 in the presence of an excess of dithizone.

The method of Cowling and Miller (20) is much more promising since it enables the determination of 5-30 micrograms of zinc.

Zinc can be determined by Walkley's Polarographic Method (97). And it can also be determined by a photometric method. This method is essentially that of Cowling and Miller (20), but modified to ensure complete extraction of the zinc from the siliceous residue. A.O.A.C. (3) have used another method for the determination of zinc in plant

tissues. They determine the light transmission of the solution by a photoelectric colorimeter with a 535 or 540 mu filter. The content of zinc is found from the standard curve.

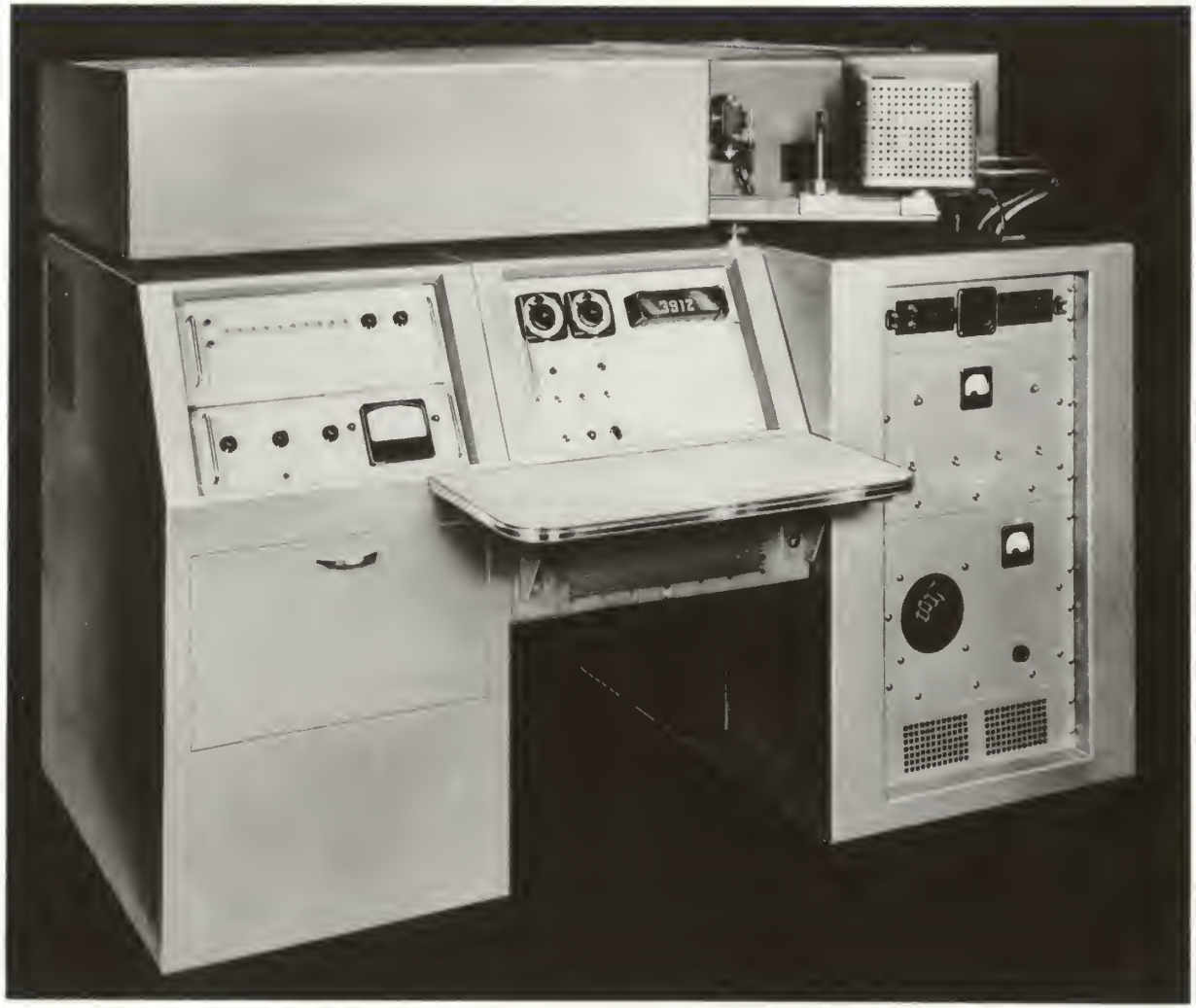
EXPLANATION OF PLATE I

Photograph of Direct Reading Spectrometer

Courtesy, Jarrel-Ash Co., Newtownville 60,

Massachusetts.

PLATE I



EXPLANATION OF PLATE II

Photograph of Atomic Absorption Spectrometer

Courtesy, The Perkin-Elmer Corporation, Norwalk,

Connecticut.

PLATE II

ATOMIC ABSORPTION ANALYSIS

Atomic absorption analysis is now easier and faster with the Model 303 atomic absorption spectrophotometer than ever before. It brings enormous improvement to the measurement of a wide range of elements. By substituting the versatile, proven instrumental method for the tedious and time-consuming methods of wet chemistry, or for interference-prone analyses by flame photometer or emission spectrometer. Drastic reductions in cost per analysis have been realized and substantial benefits in the form of higher precision and accuracy are additional.

Atomic absorption, though not a new technique, has been efficiently exploited only within the last few years. It superficially resembles flame photometry, in which metals are excited in a flame and their characteristic emission wave lengths measured. However, even metals most adaptalbe to flame photometric analysis only emit energy from a small fraction of the atoms present in the flame; the rest remains in the "ground" or unexcited state, in which they are capable of absorbing energy of the same wave lengths the excited atoms emit.

The instrumentation used in atomic absorption spectrophotometry is relatively simple. Energy of the wave length absorbed by the element to be analyzed is provided by a source lamp whose emitting cathode is made of that element. This energy is passed through a flame in which the sample is vaporized and then through a grating monochromotor

where the desired wave length is isolated from adjacent elemental emission lines.

The intensity of the energy of this wave length remaining after passage through the flame is compared with that emitted by the source, providing a direct measure of the concentration of the analyzed element in the flame and in the original sample.

Mechanism. A wide variety of source lamps can be quickly and easily mounted in the source compartment of the Model 303. There is no need of mechanical or optical adjustment. Lamp replacement takes only seconds. Energy from the source, consisting of the narrow wave length emission lines of its cathode metal, is divided by a rotating chopper into two beams, or reference beam and sample beam. The sample beam passes through the vaporizing burner into which the liquid sample is introduced. The two light beams are recombined by a semitransparent mirror, and the desired analytical wave length for the metal to be analyzed is isolated from all other energy in the light beams by a grating monochromotor. The extent to which the selected emission wave length of the source lamp is absorbed in the flame is measured by a photomultiplier tube in combination with a precise photometer that yields a digital reading corresponding directly to the concentrations of the element under analysis.

Atomic absorption is widely applied in the fields of biomedical, agricultural, metallurgical, industrial,

petroleum, mining, and geology.

With the Model 303 Atomic Absorption Spectrophotometer, one can analyze for:

a. Calcium and magnesium in biological samples.
b. Low-level sodium in the presence of high concentrations of calcium and other alkali metals.
c. Parts-per-million levels of zinc and magnesium without interference from other elements.
d. Trace metals in petroleum products.
e. Accurate alloy analyses.
f. Parts-per-million levels of semi-metals such as arsenic, selenium and tellurium.
g. And many other determinations once so difficult they were considered impractical or impossible on a routine basis.

Among the metals and other elements one can detect and measure with this instrument in parts-per-million to parts-per-billion quantities. The metals and other elements detected include: aluminum, antimony, arsenic, barium, beryllium, bizmuth, cadmium, calcium, cesium, chromium, cobalt, copper, gallium, gold, indium, iron, lead, lithium, magnesius, manganese, mercury, molybdenum, nickel, palladium, potassium, platinum, rhodium, rubidium, selentium, silver, sodium, strontium, tellurium, titanium, thallium, tin, vanadium and zinc.

Analysis with the Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer is not only sensitive but it is

fast, simple, precise, accurate, free from interference and stable too (106).

Fast: The determinations for an element can be set up, standardized and ready to run a series of tests in half an hour or less. Each subsequent measurement takes only seconds.

Simple: The repetitive measurements are as easy as inserting an aspirating tube in the liquid sample, turning a dial to balance a meter, and rending a digital value from an indicating counter.

Precise: The typical analyses show coefficients of variation less than 1 per cent of the amount present.

Accurate: The analyses are fully as accurate as the standard samples used for calibration.

Free from interference: The interference effects, common to other instrumental or wet-chemical analyses, are almost totally absent in determinations with the Model 303. Presence of elements other than the one to be measured has little or no influence.

Stable: The fluctuations in source intensity, detector sensitivity and electrical characteristics are automatically cancelled by use of a double-beam optical system.

SUMMARY

To facilitate fertilizer investigation, it is necessary to determine mineral constituents already present in the soil in an exchangeable form. This may be accomplished through soil tests or by plant analyses.

There are two methods of soil testing: Complete soil analysis and partial soil analysis. In partial soil analysis the soil is extracted with a strong acid--usually HCl of constant boiling point. Extraction with weak acid and with water and soil solution are also proposed.

Plant analyses cover both tissue and foliar testing. The age of leaf and the environmental conditions under which the plant is grown has a great effect on foliar diagnosis. The physiological position of the leaf is equally important.

The nutritional status of a plant, or plant part, is an integration of all the factors that have influence on the uptake of nutrients at the time of sampling. Such factors may include a particular plant, time of sampling, climate, management, quantity of elements added and the nature of the soil which are believed to influence nutrient uptake.

In leaf sampling one can separate the petioles and analyze the blades only, and may remove the midrib also. Extraction of dry leaf tissue with hot water or alcohol can be accomplished. But for the determination of the nutrient status of crops and the soils in which they have grown, it

is customary to analyze the entire leaf, after drying at a low temperature (70° C.), for the total quantities of the various nutrients.

The most suitable leaf for sampling is the oldest leaf that has remained healthy and functioning during the sampling period, viz. on a corn stalk, the third basal leaf is sampled.

Leaf analysis is considered an index of the nutrient status of both the plant and soil because the absorbing power of the roots regulates in part the concentration of salts in the leaves. Leaf analysis presents a picture of soil saturation at the time of sampling.

All of the plant nutrients are determined by various chemical methods. Nitrogen is determined in soils by one of the many modifications of the Kjeldahl method. Very fine grinding in a ball-mill ensures complete digestion in similar types of soils. Due to the time involved in the analysis, not many samples can be analyzed in a given period.

Colorimetric photometry and emission spectroscopy are analytical procedures which are rapid enough to analyze about 200 samples a day. Atomic absorption analysis is in practice now with the atomic absorption spectrophotometer. The method is sensitive, fast, simple, precise, accurate, free from interference and stable. Atomic absorption is widely applied in the fields of biomedicine, agriculture, metallurgy, petroleum, mining, geology and industry.

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A COMPARISON OF FOLIAR AND SOIL TESTS TO DETERMINE MINERAL DEFICIENCIES

by

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The objective of this study is to review all diagnostic methods designed for the purpose of estimating the fertility status of soils and the nutritional requirements of crops. These methods are to be regarded as complementary and not competitive. The determination of mineral constituents of plants is of importance in connection with many fertilizer investigations.

The objective of this report was to compare the foliar versus soil tests to determine mineral deficiencies in agronomic as well as horticultural crops. The predetermination of plant nutrients is necessary for successful cropping.

As early as 1804 plant analysis work was begun, but most has been done in recent years. It is necessary to make a sufficient number of crop analyses to ascertain the mineral requirements of each crop. The amounts removed by crops could be returned to the soil by adding the requisite amounts of the minerals by means of chemical fertilizers.

Mineral constituents already present in the soil in an exchangeable form can be found through soil tests and plant analysis. Soil testing is done in two ways: complete soil analysis and partial soil analysis. In partial soil analysis soil extraction is carried on with strong acids--usually HCl of constant boiling point. Extraction of soil with weak acid and with water and soil solution are also possible.

Plant analysis is also accomplished in two ways -- tissue testing and foliar analysis. The age of leaves, and environmental conditions under which the plant is grown, have a great effect on foliar diagnosis. The physiological position of the leaf must be considered. The particular plant, time of sampling, climate, management, quantity of elements

added and nature of the soil are the major factors influencing nutrient uptake and which ultimately affect the nutritional status of a plant.

Leaf analysis is important as it is considered an index of the nutrient status of both plant and soil. Leaf analysis gives an idea about the soil minerals saturation particularly at sampling time.

Sampling of the oldest leaves which remained healthy and functioning during the sampling period, gives accurate determination.

Determination of nitrogen in leaves and soil samples is commonly carried on by the Kjeldahl method. There are many modifications of this method.

All other mineral nutrients can be determined by various chemical methods, some of which require much time. Thus, a small number of samples can be analyzed in a given period of time; there are also many other limitations in these tests. Colorimetric photometry and emission spectroscopy have simplified analytical procedures. Both are efficient methods for analytical work.

The atomic absorption spectrophotometer is a new device which has recently been introduced. This instrument can be used to analyze for numerous elements. It has brought tremendous change in the rapidity of analytical work. In addition the method is sensitive, simple, precise, accurate, free from interference and stable.