8. Plant tissue testing for micronutrient deficiencies and toxicities

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Summary

The relationship between nutrient concentration and yield, when properly used, is a powerful tool for diagnosing the nutritional status of annual crops for B, Cu, Mn, Mo, Zn and occasionally Fe. Imbalance between P and Zn may affect interpretation of plant Zn data at high levels of P. Also, lack of adequate field calibration, especially that involving recently matured leaves, within geographical regions at various yield levels makes the interpretation of data for some crop-element situations difficult. Mobility of elements in plants during growth should be considered when selecting tissues for analysis. Although there undoubtedly will be exceptions, fertilization of most annual crops in the year of diagnosis is unlikely to be based on plant analysis. Successful use of plant analysis for diagnosing the micronutrient status of plants demands careful attention to plant sampling, processing of samples, and laboratory techniques. These aspects and problems with calibration and interpretation of data are discussed in detail. Sap tests would appear to have only a small role to play in diagnosis of field micronutrient problems, but they may be of assistance in studying Mn toxicity and deficiency.

Introduction

Plant analysis is a procedure by which the nutritional requirement or status of an element, an inorganic fraction of the element, or some related organic of an element, an inoragnic fraction of the element, or some related organic compound or enzyme activity that is associated with the metabolism of the plant. Plant analysis involving the micronutrients B, Cu, Fe, Mn, Mo and Zn is generally believed to be more useful for orchard or perennial crops than for annual crops [89].

In this review general principles and methodology will be discussed, but the agronomic emphasis will be placed on micronutrient analyses for B, Cu,

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Fe, Mn, Mo and Zn and the production of annual crops. Analytical data for micronutrients are usually expressed in terms of ppm or micrograms of element per gram of oven-dried tissue ($\mu g/g$).

Traditionally, plant analysis is used: (1) to make fertilizer recommendations for the current crop; (2) to identify causes of poor growth, due to either deficiencies or toxicities, under field conditions; (3) to identify possible problems associated with introduction of new crops into an area; (4) to evaluate the effectiveness of fertilizer programs; (5) to survey the nutrient status of a crop within a region; (6) to complement soil test programs; and (7) to gain an understanding of interactions among elements. Plant analysis also has an important role to play in comparing nutrient utilization by different cultivars and species. Siddiqi and Glass [94] proposed that a utilization quotient, defined as biomass per unit amount of nutrient present in biomass, should be used in such comparisons.

Because of the shortness of the growing season for annual crops, the value of plant-analysis programs for fertilizing the current crop is often questioned. However, several successful uses of the technique with micronutrients have been reported [28, 29, 32].

Goodall and Gregory's classical treatise [33] on plant analysis and a number of other general reviews provide useful background reading about this technique for evaluating plant health [2, 9, 17, 18, 20, 44, 48, 55, 56, 62, 89, 98, 105, 106, 107, 112, 113].

Theory of plant analysis

The underlying assumption behind the use of plant analysis as a diagnostic tool is that there is some relationship between levels of chemical constituents in the plant and the health of plants. The literature is not always unequivocal as to the desirable constituent to determine, the plant part and time to sample, and the meaning of the analytical data obtained. This uncertainty reflects partly the complexity of the problem and partly, for many plant species, the lack of data from suitable designed experiments.

Relevant chemical constituents

Total concentrations of B, Cu, Fe, Mn, Mo and Zn in plant tissue are normally used for diagnostic purposes. However, "active" Fe fractions, enzyme activities associated with an element, and ratios of elements are also used.

The concept and literature concerning an "active" Fe fraction and the uncertainty about the effectiveness of using total Fe for diagnostic purposes were discussed by Goodall and Gregory [33] over 35 years ago, but the issues are not yet resolved [81, 111]. A new technique (see Table 1) based on the quantity of Fe^{2+} which reacts with O-phenanthroline was found, unlike total Fe, to differentiate between the chlorotic and green leaves of rice and

Table 1. Comparison of total and O-phenanthroline-extractable Fe as criteria for separating healthy (green) and iron-deficient (chlorotic) rice plants at five sites [50]

Site	Total		O-Phenanthroline	
	Green	Chlorotic	Green	Chlorotic
	Fe, ppm			
1	135	260	51	27
2	170	200	55	29
3	140	220	53	29
4	130	270	50	34
5	160	260	52	30

possibly other crops [50]. An attractive feature of this test is that washing of leaves prior to analysis is not needed. Hydrochloric acid was previously reported to remove "active" Fe fractions from various plants, including soybeans [23, 33]. Peroxidase activity, rather than total Fe, has also been employed to diagnose Fe deficiency [7]. Iron deficiency in corn reportedly can be diagnosed by a rapid field test for peroxidase activity [8].

Many cases of Zn deficiency in the field are associated with high levels of soil P. Phosphorus-induced Zn deficiencies in plants are usually attributed to plant-dilution effects, to restricted translocation of Zn, or to an imbalance of P and Zn [82], but they may also involve a P toxicity [61]. Inactivation of Zn due to an imbalance of P and Zn could affect the efficacy of total Zn analyses in plants with high levels of P. Andrew et al. [3] concluded that normal response curves can be used for the determination of critical Zn concentrations provided samples with high P values are discarded. Gibson and Leece [31] found that total Zn in corn plants well supplied with P fertilizer was similar in both Zn-deficient and healthy plants. They proposed use of leaf carbonic anhydrase, but not ribonuclease or adolase activities, as an index of "active" Zn in corn. Various measurements of enzyme activities have been proposed for measuring the Fe, Cu, Mn, Zn and Mo status of plants [56]. Under certain conditions ratios such as P/Zn [12] and Fe/Zn [71] were found to be more effective than total Zn for diagnosing Zn deficiency in beans and corn, respectively. Shaw [93] suggested that the formation of red zinc dithizonate in nodal tissue of corn offered promise as a rapid field test for Zn.

For Mn, quick sap tests, which involve rapid semiquantitative analysis of petiole or stem sap under field conditions, were described by Nicholas [72]; these tests can be used for determining both deficiency and toxicity levels of Mn in selected horticultural crops. Syltie et al. [102] subsequently found that the sap of the midrib from a corn leaf below and opposite the ear leaf at early tassel and the sap of the petiole of the youngest mature leaf of soybean at the early pod stage can be analyzed for Mn to diagnose Mn adequacy. The possibility of using sap analysis, including that for Mn, to select plant material for regular foliar analysis has also been advocated [63]. With intensification of agriculture in tropical regions with acid soils, a suitable

sap test for Mn toxicity may prove to be an effective screening tool. Scaife and Bray [92] reviewed the role of quick sap tests for controlling the nutrient status of plants.

Nutrient level and plant growth

Knowledge of the relationship between nutrient concentration and yield is essential for interpretative purposes and for selection of the most suitable tissue for analysis. A schematic representation, based on that given by Smith [98] and Ulrich [107], for such a relationship is given in Figure 1. AB is a zone in which yield increases as nutrient concentration decreases at severe levels of deficiency. The problem of the so-called Steenbjerg or Piper-Steenbjerg effects occurs in this zone [58, 100]. Bates [9] referred to curves with AB zones as being "C-shaped." BC is a zone of large increases in dry matter with small increases in nutrient concentration and is equivalent to Ulrich's [107] "deficient" zone or Macy's [64] "minimum percentage." CD is a zone in which the nutrient concentration increases as yield increases at a proportionately slower rate; this zone corresponds to Macy's "poverty adjustment" [64] or Ulrich's [107] "transition" zone. DE is a zone in which no increase in yield is observed in spite of large increases in nutrient concentration; it corresponds to Macy's region of "luxury consumption" [64] or Ulrich's "adequate" zone [107]. EF is the "toxic" zone in which yield progressively decreases as nutrient concentration increases.

C-shaped curves [9] cause problems in interpretation of plant analysis

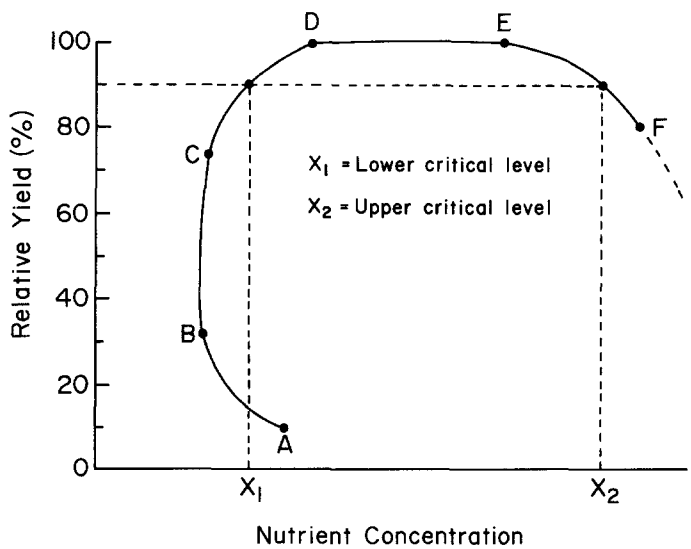

Figure 1. Schematic representation of the relationship between yield and nutrient concentration and of critical value concepts based on reports of Smith [98] and Ulrich [107].

data. Two distinctly different types of these curves have been reported: (1) those associated with low yields due to a deficiency of the element required [100]; and (2) those associated with an element not present in minimum amount required when another element causes a "Liebig Law of the Minimum" effect [104]. Problems due to the first type may be identified by sampling recently matured leaves or plant tissue soon after the appearance of symptoms [9]. Reported causes of this anomaly may be delayed senescence [87], stimulation of carbohydrate production which causes a dilution effect [95, 100], accumulation of a toxic level of another element at a low level of the element in question [70], and reduction in number of plant sinks such as tillers or grains in cereals [25]. The second type of situation is associated with growth responses caused by application of a second deficient element [104]. The possibility of complications arising from multinutrient deficiency must always be suspected with analytical data from severely deficient plants.

The ideal plant analysis situation is to arrange sampling procedures to eliminate the possibility of working in zone AB. Sharp breaks in the "transition" zone CD and in the "toxic" zone EF with reproducible critical values little affected by sampling time make diagnosis easier. Plant analysis is of limited value unless adequate research is done to provide a basis for interpretation and for selection of suitable sampling techniques, preferably with the genotypes of interest. Cultivars of many species differ greatly in their susceptibility to micronutrient deficiencies and toxicities [120], but information on intraspecific variability in critical values is limited. Corn genotypes vary in the critical level indicating Zn deficiency [65], and cultivars of wheat, cotton, and soybeans display different critical levels for Mn in the toxicity range [27].

Various terms are employed to describe quantitative or semiquantitative relationships in plant analysis. Lower and upper critical values represent nutrient composition levels in zones CD and EF, respectively. Ulrich and Hills [106, 107, 108] and Ohki [74] associate these lower and upper values with yield reductions of 10%. Other researchers define the lower critical level as being that nutrient concentration at which the element is barely above the point of limiting growth [98], the level at which a growth stress may be expected to occur [67], the level corresponding to maximum growth under a given set of conditions [70], or the nutrient concentration which is just deficient for maximum growth [105].

Dow and Roberts [21] in agreement with Smith [98] favored the use of a critical nutrient range, rather than a single critical nutrient concentration, since it is difficult to establish a single point experimentally and a single value may vary under different conditions. Although Ulrich and his associates [108] frequently give a point in zone CD (see Figure 1) as the critical value, that school of researchers recognizes that a narrow range more truly represents the situation [105]. Leaf composition values are also expressed as a series of ranges such as deficient or showing deficiency symptoms, low,

normal or sufficient, high, and excess or showing toxicity symptoms [18, 56, 89, 114]. Concentration values within ranges established under different conditions may vary considerably.

The methodology for determining critical values for annual crops is now well established, but each element/crop situation must normally be studied separately. Such studies evaluate the extent to which the plant part analyzed, the time of sampling, and the genotype affect the critical values. Crops are first grown under greenhouse conditions in nutrient solutions or in a suitable soil with different levels of the nutrient under study [75, 106]. Critical tissue data are determined at this stage by relating yield of dry matter, often expressed as percentages of maximum yield, to nutrient concentration. Specific values may be obtained by drawing curves to fit multiple data points in the "transition" and "toxic" zones [75], or by use of regression [10] or the Cate and Nelson overlay techniques [16, 91] on the experimental data.

As a followup to the greenhouse work, the critical values should be checked in field experiments suitably designed to determine the responses of the portion of the plant that is of economic value to the element in question. The part of the plant sampled in the field study is normally selected on the basis of the greenhouse study. Determination of critical values in the "transition" zone is usually stressed in the field study.

Selection of homogeneous field sites is much more difficult for micronutrient work than for P and N studied. Consequently, field data from many experiments often show no response to the micronutrient or have a high coefficient of variability. The heterogeneity also presents a problem with using unknown plant samples for routine analysis; inadvertent mixing of samples with variable composition in a composite sample may result in masking of a possible deficiency. To overcome this effect, Gartrell et al. [29] suggested that the bulk sample for diagnosing Cu deficiency in wheat should be taken from 10 identical plants from the smallest, most uniform patch of soil possible. Such a sampling technique is probably appropriate for the diagnosis of a specific problem, but it makes extrapolation of findings to entire fields difficult.

An excellent series of studies by Ohki [75, 77, 78, 79], in which critical values for Mn in soybean production were determined, can be consulted for additional insight into calibration for plant analysis. Another useful study illustrates greenhouse techniques for determining critical tissue concentrations of B, Cu, Fe, Mn, and Zn in cassava [39]. Data pertaining to critical nutrient levels have also been developed by analysis of plants showing characteristic deficiency symptoms; this approach is not as satisfactory as the previously described experimental approach [105], but for an element such as Mo it may be of some value.

Tissue selection and time of sampling

When plant analysis is used for making diagnoses or fertilizer recommendations, a part of the plant, usually recently matured leaves, is preferred for

determining B, Cu, Fe, Mn, Mo, and Zn [17, 105]. However, a few researchers favor whole-plant analysis at the boot stage for crops like wheat, oats, and barley [67, 114]. Some criteria that should be considered in the choice of type of tissue are as follows:

1. The breaks in the yield-nutrient concentration curve between the "adequate" zone and the "deficient" and toxic" zones should be sharp. Few definitive studies have been made which justify the choice of tissue for micronutrient analysis in tropical annual crops.

   Loneragan et al. [59] concluded that younger and older leaves would be clearly superior for diagnosing the nutrient status of immobile and mobile elements, respectively. Thus young tissue, rather than old leaves or whole plants, should be used for diagnosing the B status of plants. However, for the elements Fe, Mn, Zn, Cu, and Mo, classed under certain circumstances as being intermediate in mobility [15], the problem is more complex.

   Young blade tissue was efficacious for indicating both conditions of deficiency and toxicity for Mn in soybeans [75] and Zn in cotton [74], even though the toxic accumulations of the elements were much larger in older leaves. However, in a subsequent field study, Ohki et al. [78] found that the transition zone for Mn deficiency in soybeans broadened with a delay in sampling; the critical value also increased late in the season and sampling blade 2 at 9 weeks was favored for diagnosis.

   The youngest fully expanded leaf of sorghum at the boot stage [73] and of wheat from the seedling stage to senescence [29, 30] were superior to older leaves for diagnosing Mn and Cu deficiencies, respectively. The B level in the youngest mature leaf of sunflower at flowering was closely related to the percentage of deformed heads suffering from B deficiency [10]. Iron is generally considered relatively immobile in plants, and young tissue must be analyzed for diagnostic purposes [111].

   Some micronutrient deficiencies such as Zn deficiency in corn [83] and Fe deficiency in flax (*Linum usitatissimum* L.) [80] are often associated with cool or excessively wet conditions early in the season, but subsequently the plant grows out of the deficiencies as availability of soil micronutrients increases late in the season. Calibration studies using plant analysis data from such experiments are difficult, and the likelihood of a response to a micronutrient fertilizer is probably dependent on the yield potential and the length of the deficiency period.

2. The likelihood of Steenbjerg effects [100] developing and resulting in tissue concentration values greater than those associated with a deficiency must be minimized. Bates [9] considered that this problem could be reduced if sampling were restricted to plants with newly

developed symptoms.

3. The part of the plant that is sampled should preferably be one for which the critical concentration value is little affected by sampling time. Such a requirement is more difficult to attain with a fast-growing annual plant than with a perennial plant [98]. Critical concentration levels for whole plants, however, change during growth of cereals [60, 104]. Grain formation and lack of production of young, recently matured leaves in determinate crops with sexual stages present a sampling problem late in the growing season.

   Melsted et al. [67] provided data showing how leaf position and time of sampling of corn around tasseling influenced the levels of Mn, Fe, B, Zn and Cu. The effects, which probably were measured in the zone of "adequacy," varied according to the element.

4. Ease of sampling should be another factor considered in tissue selection. The corn ear leaf, or an adjacent one, which is customarily and easily sampled at silking or tasseling, is frequently analyzed for micronutrients [47, 68, 85]. The efficacy of this leaf for diagnosing micronutrient deficiencies, especially when a deficiency of Zn occurs early in the season, is questionable. Ohki et al. [76] suggested that leaf 3 or 4 might be superior to the ear leaf for diagnosing the Zn status of corn. From an analytical point of view the analysis of one tissue for most essential macro- and micronutrients is advantageous. Selection of whole plant samples for analysis of small grains also seems to be partly related to ease of sampling and convenience.

Preparation of samples for analysis

Attention must be paid to the following points during sample preparation: (1) respiration losses must be avoided during transportation of tissue to the processing center, (2) adequate subsampling techniques must be used both before and after grinding, (3) samples must be effectively ground to the required particle size, and (4) effective drying and storage techniques must be employed. Contamination must be guarded against at all stages. Sample preparation has been frequently discussed [17, 43, 44, 48, 69, 89, 96, 98, 101].

1. Any samples showing evidence of rotting at the time of processing should be discarded [57]. Most samples that cannot be processed within approximately 4 hours generally should be loosely placed in polyethylene bags, transported in an ice chest, and kept refrigerated until cleaning and oven drying can be done [17].
2. Not more than about 100 g of tissue can be finely ground conveniently. Subsampling of dried tissue before grinding tends to increase greatly the subsampling errors because of separation of veinal and interveinal tissue. If necessary, fresh samples can be cut

with stainless steel scissors or knives, uniformly mixed, and then subsampled by quartering. The ground, dried material must also be mixed uniformly before taking subsamples.

3. Many currently recommended methods of analysis require from 0.5 to 3 g of ground plant material. Material for analysis should be ground to pass sieves with either 0.8-mm or 0.4-mm openings (20- or 40-mesh, respectively); the finer material is preferable with samples in the 0.5 to 1 g range. Stainless steel Wiley mills are often used [36], and care must be taken to ensure that all the plant tissue passes through the screen. Plant material is heterogeneous, and considerable time is sometimes required to comminute the fibrous tissue. In one study, fractions of different particle sizes from ground samples contained different nutrient concentrations, but the effects differed among species [97]; this indicates that both fine grinding and careful mixing are essential.
4. Enzyme activity is usually stopped in fresh plant tissue by heating in forced-air drying cabinets, preferably supplied with filters to remove dust and maintained at 60°–70°C for 24 to 48 hours. Samples should not be packed tightly in containers or within dryers or allowed to become scorched [88]. Blades normally dry much easier than stems or petiole tissue which frequently must be cut into small pieces. Plant tissue is hygroscopic, and the finely ground powder must be redried after grinding. Drying most ground plant samples at 85°C for 12 hours would appear to be adequate for most analytical purposes.

Chemical analysis of plant tissue

Contamination problems

An understanding of likely causes of contamination is essential for increasing the efficacy of any plant analysis program, particularly one involving micronutrients. Great care must be taken to (1) remove any surface contamination; (2) avoid contamination during the collection, drying, grinding, and storage of plant tissue; and (3) avoid contamination during the analysis.

1. Any surface contamination likely to interfere with the analyses must be removed soon after the relevant tissue has been collected and before it is dried or wilted. Washing tissue requires the ready availability of distilled and/or deionized water. If a knowledge of total concentration of Fe in the plant is required, washing of plant tissue is essential. Many researchers have shown that inflated Fe values are obtained if tissues are not washed [4, 26, 37, 41, 51, 52, 99, 103, 110]. However, results of one recent study with nonsprayed soybeans grown both out-of-doors and in a greenhouse suggest that measurement of the "true" Fe content of leaves is difficult even with washing

[110]. According to Mitchell [69], an effective test for soil contamination of plant samples is to analyze for Ti, which is not normally absorbed in appreciable amounts by plants.

Soil contamination is removed much more easily than are micronutrient sprays adhering to tissue [4, 99]. Detergent alone appears to be as effective as HCl or HCl-detergent mixtures for removing soil (14), but HCl-detergent washes are recommended if plants have received alkaline sprays containing micronutrients [111]. However, such sprays are difficult to remove even with acid-detergent [4, 99] or EDTA-detergent washings [103]. If detergents are used for washing, care must be taken to avoid contamination with any element of interest.

Small or negligible losses occur from many tissues during washing procedures of short duration [4, 5, 13, 37, 52]. However, when apple leaves were soaked in 1 *N* HCl for 10 minutes to remove strongly held Zn-spray material, elements such as K, Mg and Mn were apparently lost in the wash water [84]. An effective washing procedure was proposed by Chapman [17] to remove soil.

Most research has shown that, provided reasonable care is taken to brush off any adhering soil from plant tissue, washing has little effect on unsprayed plant tissue values for Cu, Zn, Mo, and B [5, 13, 14, 26, 37, 52]. However, washing occasionally has slightly reduced the Mn concentrations in plant tissue, presumable by removing contamination [14, 26]. Experience, knowledge of the local situation, and the value of Fe data are factors influencing the decision of whether to wash tissue or not.

2. Care should be taken that utensils used for harvesting and preparing samples for analysis do not introduce contamination. Some paper bags contain B and could cause contamination [117]. Drying ovens should preferably be of stainless steel construction or painted with a high quality epoxy paint; galvanized trays must not be used because of likely Zn contamination. Mechanical chrome-plated [53, 111], agate [43, 86], and stainless steel mills [5, 36] are recommended for grinding; brass and ordinary steel parts must not be allowed to come in contact with plant material. Also, storage of ground plant material in polyethylene bags or glassine-lined bags, rather than in glass containers, is occasionally preferred to decrease contamination [86, 95]; glass bottles, however, are commonly used.
3. Extreme care is needed to avoid accidental or systematic introduction of foreign elements in the course of various analytical operations [86]. As far as possible reagents should be stored in polyethylene containers. Acid washing of glassware followed by rinsing in distilled or deionized water is essential. Johnson and Ulrich [43] recommended washing glassware in warm 3 *N* HCl, rinsing in succession with

several small portions of redistilled water and 10% (w/v) $(NH_4)_3$ EDTA (pH 8), and finally washing with redistilled water. Washing glassware used in micronutrient studies with 20% (v/v) HNO_3 [49] and 0.5 *N* acetic acid or 0.5 *N* HCl [88] followed by rinsing with tap water and successive portions of distilled and/or deionized water has also been reported.

Rubber used in tubing, stoppers, clamps of shakers, etc., can cause Zn contamination, and its use should be avoided [69, 86]. Rubber bulbs attached to transfer pipettes can cause contamination with Zn, Cu, and Fe [5]. Pyrex or Kimax glassware contains B, and any solutions used in analyses for this element should be stored in polyethylene bottles [86]. In separate studies, dust from linings of furnaces used in dry-ash digestions was found to cause both B [116] and Zn and Al [5] contamination. Lining the inside of the furnace with stainless steel sheeting eliminated the problem for Zn and Al [5]. Corrosion of metal surfaces within the laboratory can be particularly troublesome. Copper or brass fittings should be replaced or coated with an epoxy paint or resin; basic salts formed on copper surfaces can be readily diffused as a fine dust [88]. Pinta [86] suggests that all personnel working with trace elements should be warned of all the risks of contamination. Glassware used for trace element analyses should be reserved for that purpose. Colorimetric analysis for phosphorus frequently contaminates laboratories with Mo, and such work should be done away from a micronutrient laboratory doing Mo analyses. Every effort should be made to control dust in the laboratory.

Careful attention should be paid to the distilled-deionized water system [24]. Rubber, brass, bronze, or copper surfaces should not come in contact with purified water, and polyethylene storage containers should be used.

Precision and accuracy

Considerable effort is needed to ensure that good precision and accuracy are obtained during chemical analysis. Precision, representing operator or random laboratory errors, can be easily calculated as a coefficient of variation by doing analyses on subsamples of a relevant sample. However, this gives no estimate of method bias or accuracy [66].

Measurement of accuracy is best obtained by including a "standard" control sample in each batch of unknown samples. This task is simplified by the availability of plant samples as Standard Reference Materials (SRM) from the U.S. National Bureau of Standards. SRM 1570 (spinach), SRM 1571 (orchard leaves), SRM 1573 (tomato leaves), and SRM 1575 (pine needles) have been extensively used, and data of interest for the first three samples are given in Table 2. The SRM plant tissues described in Table 2 contain more than 10 times the reported critical amounts of Cu in young wheat leaves at

Table 2. Analytical data provided by the U.S. National Bureau of Standards for Fe, Mn, Zn, Cu, Mo and B for three plant standards.

Material	SRM No.*	Element†					
		Fe	Mn	Zn	Cu	Mo	B
		μg/g					
Spinach	1570	555 ± 20	165 ± 6	50 ± 2	12 ± 2	a	(30)
Orchard leaves	1571	300 ± 20	91 ± 4	25 ± 3	12 ± 1	0.3 (± .1)	33 ± 3
Tomato leaves	1573	690 ± 25	238 ± 7	62 ± 6	11 ± 1	b	(30)

*The supply of SRM 1571 has unfortunately become exhausted but a new citrus leaf sample, SRM 1572, became available during 1982.
†"a" and "b" indicate that no information from National Bureau of Standards (NBS) was provided; however, data reported in the literature [49] give values of 0.3 ± 0.1 and 0.65 ± 0.10 and 0.62 ± 0.04 μg/g, respectively. Numbers in parenthesis were noncertified NBS values.

tillering [29] and of Fe in corn leaves at silking [67]. Cost of SRMs is now exceeding $100/75 g, and this may limit their use on a routine basis in some laboratories.

Several laboratories or groups of analysts may conveniently pool their resources for preparation and standardization of a suitable plant standard. Use of a carefully analyzed control, such as an SRM sample, is efficacious in the calibration of secondary standards. Interlaboratory exchanges of suitable samples have a valuable role to play in the evaluation of new methods and in quality control [40, 45, 54, 115].

Method of analysis

Chemical methods for determination of total elements based on emission spectroscopy, atomic absorption spectrophotometry (AAS), inductively coupled argon plasma emission spectroscopy (ICAP), and colorimetry require that the organic matter be removed and the elements be solubilized before analysis. Both wet and dry digestion techniques are commonly used. However, Baker and Greweling [6] reported that Cu and Zn analyses made on 0.1*M* EDTA extracts of sorghum and other crops yielded values comparable to those obtained after dry ashing. Choice of methods depends on such factors as convenience, safety, available equipment, and elements to be analyzed.

Where large numbers of plant samples are analyzed for diagnostic purposes, there is a tendency to favor dry-ashing techniques. One advantage of the dry-ashing technique is that a separate digestion is not required for B, which is difficult to analyze by wet-digestion methods because of possible contamination associated with use of Pyrex glassware [30] and volatilization during digestion [43].

Recommended dry-ashing techniques for plant tissue have been extensively discussed by Piper [88] and Gorsuch [34, 35]. Volatilization losses and reactions of micronutrients with surfaces of crucibles and with silica from

plants are possible causes of low recoveries with this method of organic matter removal. Gorsuch [34] concluded that considerable experience is needed in using this technique to solubilize effectively the elements in plant tissue; its application to unknown samples without first discovering its suitability is questionable. Overheating in furnaces must be avoided [34, 88]. Jones [46] recommended that the crucibles used, 15-ml high and made of porcelain, should not come in contact with walls or floors of furnaces heated to 500°C.

The availability of Al-heating blocks, which allow many 75-ml digestion tubes to be heated in a small area, has popularized wet-digestion techniques in the past decade [11]. Addition of one or two drops of kerosene and use of blocks thermostatically controlled at suitable temperatures greatly aid digestion [1]. Some digestion mixtures, discussed by Gorsuch [35], that have been used for plant decomposition are HNO_3-$HClO_4$ [1, 40, 49, 121], HNO_3-$HClO_4$-H_2SO_4 [43, 50], and H_2SO_4-H_2O_2 [119]. Nitric acid alone [38] was recently reported to be nearly as efficacious as HNO_3-$HClO_4$, the most commonly used digestion mixture for plant analysis.

Wet-digestion methods involving use of HNO_3-$HClO_4$ to destroy organic matter appear reasonably efficacious for recovery of Cu, Zn, Mn, and possible Mo, but not for recovery of Fe [49] or B [43]. Wolf [118] described a wet-digestion method for B determination involving heating of plant tissue with H_2SO_4-H_2O_2 in Vycor tubes. Gestring and Soltanpour [30] also concluded that B was not lost from HNO_3 digests heated at 90°C in Nalgene bottles.

The exact method of analysis will depend upon available equipment, number of samples to be anayzed, and cost considerations. Where large numbers of samples must be analyzed for essential micronutrients as well as for Al, P, Ca, Mg, Na, and K, ICAP techniques and dry ashing appear attractive for Cu, Mn, Zn, and B [46, 49]. However, sensitivity of ICAP techniques is poor [49] for Mo determinations at plant levels near 0.3 μg/g, a level well above that found in some Mo-deficient plants. Colorimetric techniques [22, 43] appear to be superior in such cases. Colorimetric techniques [42, 43, 118] probably are also to be preferred for plant B analyses if the AAS method, instead of ICAP or emission spectroscopy, is used for Cu, Zn, and Mn analyses. Because of difficulties with contamination and analysis, and the often-noted poor relationship of total Fe with plant Fe stress [33, 50, 110], it is questionable whether much emphasis should be placed on total Fe analysis for routine diagnostic purposes.

Difficulties with plant analysis

Both theoretical and practical difficulties restrict the applicability of plant analysis for diagnosing micronutrient problems in annual crops over wide regions. Special attention must be given to time of sampling and plant part

selected for analysis. Additional research is needed on the efficacy of using the same part for diagnosing both nutrient deficiencies and toxicities.

Although there undoubtedly will be exceptions, the prime use of plant analysis will probably be as an aid in the interpretation of field problems rather than as a routine means of making fertilizer recommendations. Micronutrient deficiencies in most areas of the world are secondary to deficiencies of N and P. This together with the cost of any routine plant analysis program, relative to the value of most annual field crops, places restrictions on the use of this technique.

If yields are extremely low because of a particular deficiency, only limited information about other elements can be gained from plant analysis. This situation is often encountered with plants growing on soils where micronutrient deficiencies are only obvious when deficiencies of N and P are rectified. Such situations are related to Liebig's Law of the Minimum [90]. For instance, Chaudhry and Loneragan [19] found that wheat when grown on a particular soil responded to Cu and Zn fertilizers only if nitrogen fertilizer was applied; the increased growth resulting from the response to N diluted the quantities of Cu and Zn to deficiency levels. Andrew [2] considered that, if a legume was not well supplied with symbiotic or mineral N, plant analysis for other elements served little purpose.

Multiple nutrient deficiencies at levels where plants respond to two or more elements, both individually and in combination, also present interpretation difficulties. Only limited research is available with annual crops showing how critical levels or ranges change in such circumstances.

Interactions between plant nutrients undoubtedly complicate interpretation of plant analysis data, and blind acceptance of individual critical values or ranges must be viewed with caution. Regression techniques have been used to refine relationships between critical values and yield [85, 109]. Bates [9] concluded that interactions become more important in the vicinity of the optimum yield. Adequate field research with specific crops in given environments is needed to evaluate the importance of interactions among nutrients and between nutrients and the environment.

References

1. Allen JE (1969) The preparation of agricultural samples for analysis by atomic absorption spectroscopy. Walnut Creek, California: Varian Techtron.
2. Andrew CS (1968) Problems in the use of chemical analyses for diagnosis of plant nutrient deficiencies. J Aust Instit Agr Sci 12, 154–162.
3. Andrew CS, Johnson AD and Haydock KP (1981) The diagnosis of zinc deficiency and effect of zinc on the growth and chemical composition of some tropical and sub-tropical legumes. Comm Soil Sci Plant Anal 12, 1–18.
4. Arkley TH, Munns DN and Johnson CM (1960) Preparation of plant tissue for micronutrient analysis. J Agr Food Chem 8, 318–321.
5. Baker DE, Gorsline GW, Smith CB, Thomas WI, Grube WE, and Ragland JL (1964) Technique for rapid analyses of corn leaves for eleven elements. Agron J 56, 133–136.

6. Baker JH and Greweling T (1967) Extraction procedure for quantitative determination of six elements in plant tissue. J Agr Food Chem 15, 340–344.
7. Bar-Akiva A, Kaplan M and Lavon R (1967) The use of a biochemical indicator for diagnosing micronutrient deficiencies of grapefruit trees under field conditions. Agrochimica 11, 283–288.
8. Bar-Akiva A, Maynard DN and English JE (1978) A rapid tissue test for diagnosing iron deficiencies in vegetable crops. Hort Sci 13, 284–285.
9. Bates TE (1971) Factors affecting critical nutrient concentrations in plants and their evaluation: a review. Soil Sci 112, 116–130.
10. Blamey FPC, Mould D and Nathanson K (1978) Relationships between B deficiency symptoms in sunflowers and the B and Ca/B status of plant tissues. Agron J 70, 376–380.
11. Blanchar RW, Rehm G and Caldwell AC (1965) Sulfur in plant materials by digestion with nitric and perchloric acid. Soil Sci Soc Am Proc 29, 71–72.
12. Boawn LC and Brown JC (1968) Further evidence for a P-Zn imbalance in plants. Soil Sci Soc Am Proc 32, 94–97.
13. Boswell FC (1972) Soybean leaf analysis of washed versus unwashed samples from boron-manganese studies. Comm Soil Sci Plant Anal 3, 243–251.
14. Bradfield EG and Bould C (1963) Leaf analysis as a guide to the nutrition of fruit crops. III. J Sci Food Agr 14, 729–733.
15. Bukovac JM and Wittwer SH (1957) Absorption and mobility of foliar applied nutrients. Plant Physiol 32, 428–435.
16. Cate RB and Nelson LA (1965) A rapid method for correlation of soil test analyses with plant response data. North Carolina Agr Exp Sta Inter Soil Testing Tech Bull No 1.
17. Chapman HD (1964) Foliar sampling for determining the nutrient status of crops. World Crops 36–46.
18. Chapman HD (ed) (1966) Diagnostic criteria for plants and soils. Univ California Div Agr Sci.
19. Chaudhry FM and Loneragan JF (1970) Effects of nitrogen, copper, and zinc fertilizers on the copper and zinc nutrition of wheat plants. Aust J Agr Res 21, 865–879.
20. Clements HF (1964) Interaction of factors affecting yield. Ann Rev Plant Physiol 15, 409–422.
21. Dow AI and Roberts S (1982) Proposal: Critical nutrient ranges for crop diagnosis. Agron J 74, 401–403.
22. Eivazi F, Sims JL and Crutchfield J (1982) Determination of molybdenum in plant materials using a rapid automated method. Comm Soil Sci Plant Anal 13, 135–150.
23. Elgala M and Maier RH (1964) Chemical forms of plant and soil iron as influenced by soil moisture. Plant Soil 21, 201–212.
24. Environmental Monitoring and Support Laboratory (1979) Handbook for analytical quality control in water and wastewater laboratories. Cincinnati, Ohio: U.S. Environmental Protection Agency.
25. Etchevers JD (1970) The influence of CCC and nitrogen on the yield and performance of wheat and triticale. M Sc Thesis, North Dakota State University, Fargo, North Dakota.
26. Floyd RA and Ragland JL (1966) Soil contamination of field-grown corn plants. Agron J 58, 460–461.
27. Foy CD, Chaney RL and White MC (1978) The physiology of metal toxicity in plants. Ann Rev Plant Physiol 29, 511–566.
28. Gartrell JW, Brennan RF and Robson AD (1979) Symptoms and treatment of copper deficiency of wheat. J Agr W Aust 20 (4th series), 18–20.
29. Gartrell JW, Robson AD and Loneragan JF (1979) A new tissue test for accurate diagnosis of copper deficiency in cereals. J Agr W Aust 20 (4th series), 86–88.
30. Gestring WD and Soltanpour PN (1981) Evaluation of wet and dry digestion methods for boron determination in plant samples by ICP-AES. Comm Soil Sci Plant Anal 12, 743–753.

31. Gibson TS and Leece DR (1981) Estimation of physiologically active zinc in maize by biochemical assay. Plant Soil 63, 395–406.
32. Goodall DW (1949) Studies in the diagnosis of mineral deficiency. V. Manganese deficiency in wheat. Ann Appl Biol 36, 26–39.
33. Goodall DW and Gregory FG (1947) Chemical composition of plants as an index of their nutritional status. Imp Bur Hort Plantation Crops (East Malling) Comm 17.
34. Gorsuch TT (1959) Radio chemical investigations on the recovery for analysis of trace elements in organic and biological materials. Analyst 84, 135–173.
35. Gorsuch TT (1970) The destruction of organic matter. Oxford: Pergamon Press.
36. Grier JD (1966) Preparation of plant material for plant analysis. J Ass Off Anal Chem 49, 292–298.
37. Guha MM and Mitchell RL (1965) The trace and major element composition of the leaves of some deciduous trees 1. Sampling techniques. Plant Soil 23, 323–338.
38. Havlin JL and Soltanpour PN (1980) A nitric acid plant tissue digest method for use with inductively coupled plasma spectrometry. Comm Soil Sci Plant Anal 11, 969–980.
39. Howeler RH, Edwards DG and Asher CJ (1982) Micronutrient deficiencies and toxicities of cassava plants grown in nutrient solutions. J Plant Nutr 5, 1059–1077.
40. Issac RA and Johnson WC (1975) Collaborative study of wet and dry ashing techniques for the elemental analysis of plant tissue by atomic absorption spectrophotometry. J Ass Offic Anal Chem 58, 436–440.
41. Jacques GL, Vanderlip RL, Whitney DA and Ellis R Jr. (1974) Nutrient contents of washed and unwashed grain sorghum plant tissue compared. Comm Soil Sci Plant Anal 5, 173–182.
42. John MK, Chuah HH and Neufeld JH (1975) Application of improved azomethine-H method for determination of boron in soils and plants. Anal Lett 8, 559–568.
43. Johnson CM and Ulrich A (1959) Analytical methods for use in plant analysis. California Agr Exp Sta Bull 766.
44. Jones JB Jr. (1972) Plant tissue analysis for micronutrients. In Mortvedt JJ, Giordano PM and Lindsay WL (eds) Micronutrients in agriculture, pp 319–346. Madison, Wisconsin: Soil Sci Soc Am, Inc.
45. Jones JB Jr. (1975) Collaborative study of the elemental analysis of plant tissue by direct reading emission spectroscopy. J Ass Off Anal Chem 58, 764–769.
46. Jones JB Jr. (1977) Elemental analysis of soil extracts and plant tissue ash by plasma emission spectroscopy. Comm Soil Sci Plant Anal 8, 349–365.
47. Jones JB Jr and Eck HV (1973) Plant analysis as an aid in fertilizing corn and grain sorghum. In Walsh LM and Beaton JD (eds) Soil testing and plant analysis, pp 349–364. Madison, Wisconsin: Soil Sci Soc Am, Inc.
48. Jones JB Jr and Steyn WJA (1973) Sampling, handling, and analyzing plant tissue samples. In Walsh LM and Beaton JD (eds) Soil testing and plant analysis, pp 249–270. Madison, Wisconsin: Soil Sci Soc Am, Inc.
49. Jones JW, Capar SG and O'Haver TC (1982) Critical evaluation of a multi-element scheme using plasma emission and hydride evolution atomic-absorption spectrophotometry for the analysis of plant and animal tissues. Analyst 107, 353–377.
50. Katyal JC and Sharma BD (1980) A new technique of plant analysis to resolve iron chlorosis. Plant Soil 55, 105–118.
51. Labanauskas CK (1966) Effects of orange leaf-washing techniques on removal of surface contamination and nutrient losses. Proc Am Soc Hort Sci 89, 201–205.
52. Labanauskas CK (1968) Washing citrus leaves for leaf analysis. California Agr 22(1), 12–13.
53. Labanauskas CK, Nauer EM and Roistacher CN (1967) Initial soil-mix and post-planting liquid fertilization effects on nutrient concentrations in Valencia orange seedling leaves. Hilgardia 38, 569–577.
54. La Bastide JGA and van Goor CP (1978) Interlaboratory variability in the chemical analysis of leaf samples. Plant Soil 49, 1–7.
55. Leece DR (1968) The concept of leaf analysis for fruit trees. J Aust Instit Agr Sci 34, 146–153.

56. Leece DR (1976) Diagnosis of nutritional disorders of fruit trees by leaf and soil analyses and biochemical indices. J Aust Instit Agr Sci 42, 3–19.
57. Lockman RB (1970) Plant sample analysis as affected by sample decomposition prior to laboratory processing. Comm Soil Sci Plant Anal 1, 13–19.
58. Loneragan JF (1975) The availability and absorption of trace elements in soil-plant systems and their relation to movement and concentration of trace elements in plants. In Nicholas DJD and Egan AR (eds) Trace elements in soil-plant animal systems, pp 109–134, New York: Academic Press.
59. Loneragan JF, Snowball K and Robson AD (1976) Remobilization of nutrients and its significance in plant nutrition. In Wardlaw IF and Passioura JB (eds) Transport and transfer processes in plants, pp 463–469. New York: Academic Press.
60. Loneragan JF, Snowball K and Robson AD (1980) Copper supply in relation to content and redistribution of copper among organs of the wheat plant. Ann Bot 45, 621–632.
61. Loneragan JF, Grove TS, Robson AD and Snowball K (1979) Phosphorus toxicity as a factor in zinc-phosphorus interactions in plants. Soil Sci Soc Am J 43, 966–972.
62. Lundegardh H (1951) Leaf analysis. London: Hilger and Watts Ltd.
63. Lynd JQ, Turk LM and Cook RL (1950) Nutrient deficiencies diagnosed with foliar analyses and plant tissue tests. Agron J 42, 402–407.
64. Macy P (1936) The quantitative mineral nutrient requirements of plants. Plant Physiol. 11, 749–764.
65. Massey HF (1971) Interstrain variation as a factor to be considered in soil and plant studies of zinc deficiency in maize. Agron Abstr p 92.
66. McFarren EF, Lishka RJ and Parker JH (1970) Criterion for judging acceptability of analytical methods. Anal Chem 42, 358–365.
67. Melsted SW, Motto HL and Peck TR (1969) Critical plant nutrient composition values useful in interpreting plant analysis data. Agron J 61, 17–20.
68. Minotti PL (1975) Plant nutrition and vegetable quality. Hort Sci 10, 54–56.
69. Mitchell RL (1960) Contamination problems in soil and plant analysis. J Sci Food Agr 11, 553–560.
70. Moore DP, Harward ME, Mason DD, Hader RJ, Lott WL and Jackson WA (1957) An investigation of some of the relationships between copper, iron, and molybdenum in the growth and nutrition of lettuce. Soil Sci Soc Am Proc 21, 65–74.
71. Nambiar KKM and Motiramani DP (1981) Tissue Fe/Zn ratio as a diagnostic tool for prediction of Zn deficiency in crop plants. Plant Soil 60, 357–367.
72. Nicholas DJD (1948) The application of rapid chemical tests to the diagnosis of mineral deficiencies in horticultural crops. J Hort Sci 24, 106–122.
73. Ohki K (1975) Manganese supply, growth, and micronutrient concentration in grain sorghum. Agron J 67, 30–32.
74. Ohki K (1975) Lower and upper critical zinc levels in relation to cotton growth and development. Physiol Plant 35, 96–100.
75. Ohki K (1976) Manganese deficiency and toxicity levels for 'Bragg' soybeans. Agron J 68, 861–864.
76. Ohki K, Anderson OE and Jones LS (1976) Residual Zn availability related to growth and nutrient gradients in corn. Agron J 68, 612–614.
77. Ohki K, Boswell FC, Parker MB, Shuman LM and Wilson DO (1979) Critical manganese deficiency level of soybean related to leaf position. Agron J 71, 233–234.
78. Ohki K, Wilson DO, Boswell FC, Parker MB and Shuman LM (1977) Mn concentration in soybean leaf related to bean yields. Agron J 69, 597–600.
79. Ohki K, Wilson DO, Boswell FC, Parker MB and Shuman LM (1978) Critical levels of manganese in soybeans. Georgia Agr Res 19(3), 18–23.
80. Olomu MO and Racz GJ (1974) Effect of soil water and aeration on Fe and Mn utilization by flax. Agron J 66, 523–526.
81. Olsen RA, Brown JC, Bennett JH and Blume D (1982) Reduction of Fe^{3+} as it relates to Fe chlorosis. J Plant Nutr 5, 433–445.
82. Olsen SR (1972) Micronutrient interactions. In Mortvedt JJ, Giordano PM and

Lindsay WL (eds) Micronutrients in agriculture, pp 243–264. Madison, Wisconsin: Soil Sci Soc Am, Inc.
83. Olson RA and Lucas RE (1964) Fertility requirements: secondary and micronutrients. In Pierre WH, Aldrich SA and Martin WP (eds) Advances in corn production, pp 285–330. Ames, Iowa: Iowa State University Press.
84. Orphanos PI (1977) Removal of zinc residues from apple leaves by hydrochloric acid and leaching of other nutrients in the process. J Sci Fd Agr 28, 643–646.
85. Peck TR, Walker WM, and Boone LV (1969) Relationship between corn (*Zea mays* L) yield and leaf levels of ten elements. Agron J 61, 299–301.
86. Pinta M (1971) Detection and determination of trace elements. London: Ann Arbor Science.
87. Piper CS (1942) Investigations on copper deficiency in plants. J Agr Sci 32, 143–178.
88. Piper CS (1950) Soil and plant analysis. Adelaide, Australia: The University of Adelaide.
89. Reisenauer HM (ed) (1976) Soil and plant-tissue testing in California. Univ California Div Agr Sci Bull 1879.
90. Russell EW (1973) Soil conditions and plant growth, 10th edn. London: Longman.
91. Sakal R, Singh BP and Singh AP (1982) Determination of critical limit of zinc in soil and plant for predicting response of rice to zinc application in calcareous soils. Plant Soil 66, 129–132.
92. Scaife MA and Bray BG (1977) Quick sap tests for improved control of crop nutrient status. ADAS Quarterly Rev 27, 137–145.
93. Shaw E (1952) A dithizone tissue test for zinc in plants. Soil Sci. 74, 479–480.
94. Siddiqi MY and Glass ADM (1981) Utilization index: a modified approach to the estimation and comparison of nutrient utilization efficiency in plants. J Plant Nutr 4, 289–302.
95. Sillanpää M (1982) Micronutrients and the nutrient status of soils. FAO (Rome) Soils Bull 48.
96. Smith CB (1956) The importance of dry weight losses in the preparation of leaf samples for analysis. Proc Am Soc Hort Sci 67, 10–15.
97. Smith JH, Carter DL, Brown MJ and Douglas CL (1968) Differences in chemical composition of plant sample fractions resulting from grinding and screening. Agron J 60–149.
98. Smith, PF (1962) Mineral analysis of plant tissues. Ann Rev Plant Physiol 13, 81–108.
99. Smith PF, Reuther W and Specht AW (1950) Mineral composition of chlorotic orange leaves and some observations on the relation of sample preparation technique to the interpretation of results. Plant Physiol 25, 496–506.
100. Steenbjerg F (1951) Yield curves and chemical plant analyses. Plant Soil 3, 97–109.
101. Steyn WJA (1961) The errors involved in the sampling of citrus and pineapple plants for leaf analysis purposes. In Reuther W (ed) Plant analysis and fertilizer problems, pp 409–430. Washington, DC: Am Instit Biol Sci.
102. Syltie PW, Melsted SW and Walker WM (1972) Rapid tissue tests as indicators of yield plant composition, and soil fertility for corn and soybeans. Comm Soil Sci Plant Anal 3, 37–49.
103. Taylor GA (1956) The effectiveness of five cleaning procedures in the preparation of apple leaf samples for analysis. Proc Am Soc Hort Sci 67, 5–9.
104. Terman GL, Noggle JC and Engelstad OP (1972) Concentration of N and P in young corn plants as affected by various growth-limiting factors. Agron J 64, 384–388.
105. Ulrich A (1952) Physiological bases for assessing the nutritional requirements of plants. Ann Rev Plant Physiol 3, 207–228.
106. Ulrich A (1961) Plant analysis in sugar beet nutrition. In Reuther W (ed) Plant analysis and fertilizer problems, pp 190–211. Washington, DC: Am Instit Biol Sci
107. Ulrich A (1976) Plant tissue analysis. In Reisenauer HM (ed) Soil and plant-tissue testing in California, pp 1–4. Univ California Div Agr Sci Bull 1879.

108. Ulrich A and Hills FJ (1969) Sugar beet nutrient deficiency symptoms. A color atlas and chemical guide. Univ California Div Agr Sci 36 p.
109. Voss RE, Hanway JJ and Dumenil LC (1970) Relationship between grain yield and leaf N, P, and K concentrations for corn (*Zea mays* L) and the factors that influence this relationship. Agron J 62, 726–728.
110. Wallace A, Mueller RT, Cha JW and Romney EM (1982) Influence of washing of soybean leaves on identification of iron deficiency by leaf analysis. J Plant Nutr 5, 805–810.
111. Wallihan EF (1977) Tissue tests for iron. In Reisenauer HM (ed) Soil and plant-tissue testing in California, pp 32–34. Univ California Div Agr Sci Bull 1879.
112. Walsh LM (ed) (1971) Instrumental methods for analysis of soils and plant tissue. Madison, Wisconsin: Soil Sci Soc Am, Inc.
113. Walsh, LM and Beaton JD (eds) (1973) Soil testing and plant analysis. Madison, Wisconsin: Soil Sci Soc Am, Inc.
114. Ward RC, Whitney DA and Westfall DG (1973) Plant analysis as an aid in fertilizing small grains. In Walsh LM and Beaton JD (eds) Soil testing and plant analysis, pp 329–488. Madison, Wisconsin: Soil Sci Soc Am, Inc.
115. Watson ME (1981) Interlaboratory comparison in the determination of nutrient concentrations of plant tissue. Comm Soil Sci Plant Anal 12, 601–617.
116. Williams DE and Vlamis J (1961) Boron analysis of plant tissue. Soil Sci 92, 161–165.
117. Winsor HW (1957) Boron contamination of soil samples collected in paper bags. Soil Sci 84, 389–394.
118. Wolf B (1971) The determination of boron in soil extracts, plant materials, composts, manures, water and nutrient solutions. Comm Soil Sci Plant Anal 2, 363–374.
119. Wolf WR (1978) Trace element analysis of wheat base-line samples. In Proceedings 10th National Conference on Wheat Utilization Research. U.S. Dept Agr, Agr Reviews and Manuals ARM-W-4, August, 1978.
120. Wright MJ, ed. (1976) Plant adaptation to mineral stress in problem soils. Proc of Workshop, Beltsville, Maryland, November 22–23, 1976. Special Publn Cornell Univ Agr Exp Sta.
121. Zasoski RJ and Burau RG (1977) A rapid nitric-perchloric acid digestion method for multi-element tissue analysis. Comm Soil Sci Plant Anal 8, 425–436.