

Table II. Phosphate and Sulfate Ions Found in Phosphorus Fertilizers by Suggested Method and by Gravimetry

Added to 10 Ml. of Superphosphate Stock Soln.			EtOH Added in Titration of		MgC ₂ O ₄ Consumed Ml.	P ₂ O ₅ , %		EtOH Added in Titration of SO ₄ ⁻² ,		SO ₄ ⁻² , %		Dev. from Gravimetric Values, %		
DCTA, ^a ml.	EDTA, ^a ml.	Buffer, ml.	PO ₄ ⁻³ , Ml.	PO ₄ ⁻³ , Ml.		Authors' method	Gravimetry	Ml.	BaCl ₂ ^a Consumed, Ml.	Authors' method	Gravimetry	PO ₄ ⁻³	SO ₄ ⁻²	
4	9	3	5	6.45 6.40 6.43	6.43	22.96	22.96	20	7.20 7.30 7.40 7.40 7.45	7.33	35.43	34.98	0.00	1.29

^a 0.1 M solution.

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PLANT TISSUE ANALYSIS

X-Ray Fluorescence Determination of Zinc in Plant Tissue

L. D. WHITTIG, J. R. BUCHANAN, and A. L. BROWN
 University of California, Davis, Calif.

X-ray emission spectroscopy has been applied to determination of zinc in a variety of plant species. Data are presented to show the lack of consistency in the intensity of the zinc emission maxima as a function of zinc concentration in plant tissue of complex and variable composition. Known amounts of zinc are added to samples and the original concentration is calculated on the basis of the response to the known added zinc.

INTERPRETATION of factors affecting intensity of emission maxima and compensation for them are of primary concern in applying x-ray emission spectroscopy to the quantitative analysis of chemical elements. Compensation for interferences can often be calculated or otherwise easily determined in relatively simple systems of limited elemental components. Evaluation and compensation for interferences in complex systems such as soils and plant tissue present a much more difficult problem.

As reviewed by Liebhafsky (9), variations in the intensity of fluorescent x-ray maxima of elements result from absorption of excitation energy and of secondary emission energy by the sample and enhancement of emission maxima of an element as a result of excitation by higher energy secondary radiation emanating from other elements in the sample. Besides these factors which affect intensity of emission maxima directly, variable matrix compositions contribute to variations in background radiation levels. Variable absorption and enhancement together with variable background levels in samples of different composition require most careful evaluation and compensation in quantitative fluorescence analysis.

Compensation for Interferences. The magnitude of absorption interfer-

ence in relatively simple chemical systems can often be calculated from known absorption coefficients, so that suitable corrections can be made (4). Alternatively, in simple systems, standard curves may be prepared in which elemental emission intensity is plotted against concentration of interfering elements (5). A commonly used technique is the addition of internal standards which are affected in the same manner as the elements being analyzed (3, 5). Suitable correction can be applied to the emission intensity of the unknown on the basis of variation in intensity of known concentration of internal standard.

An interference compensating technique employed by Kemp and Andermann (7), Andermann and Kemp (7), and Brandt and Lazar (2) utilizes scattered radiation as an internal standard in emission intensity measurements. In theory, scattered radiation of specific wave length can be selected which is affected by absorption in nearly the same way as the emitted radiation of an element being analyzed. A ratio of an element's emission intensity to intensity of the scattered radiation at the selected wave length will be constant so long as the interferences result only from instrument variability and absorption of secondary emission energy.

The ratio technique can be valid only

if interferences affect emission and scatter intensity proportionately—not in situations where scatter intensity varies independently of elemental emission intensity, or where there is variable absorption of primary excitation energy.

It would appear that any variable affecting the intensity of an element's emission maxima in a sample could conveniently be measured and compensated for by adding a known quantity of the element concerned and relating the increase in intensity obtained by the addition to that originally present. Such a technique has been applied in analysis of inorganic mineral samples (3, 10). The present investigation is concerned with instrumentation and a comparison of methods of evaluating concentration levels of zinc in tissue of various plant species. The method of analysis described combines precision and accuracy with speed suitable for routine analyses on a large scale.

Experimental

Instrumentation. A spectrograph (General Electric XRD-5) equipped with a tungsten target x-ray tube (Machlett AEG-50), lithium fluoride analyzing crystal, 0.005-inch Solar slit, and krypton-filled proportional counter detector was used. The optimum voltage

Table I. Effect of Sample Packing on Zinc Emission Intensity and Emission to Scatter Ratio

F-S		F/S	
Loose-pack, c.p.s.	Briquet, c.p.s.	Loose-pack, c.p.s.	Briquet, c.p.s.
14.5	15.0	1.29 ^a	1.29 ^b
3.3	2.5	1.06	1.05
8.1	9.0	1.16	1.17
10.8	10.8	1.22	1.21
18.5	19.5	1.38	1.36
3.5	2.7	1.07	1.05
4.7	3.9	1.09	1.07
33.5	35.3	1.62	1.62
3.6	3.0	1.07	1.06
12.6	12.8	1.25	1.24
66.5	73.1	2.29	2.31
9.5	10.2	1.18	1.18
68.0	73.0	2.30	2.30

^a 1.286 ± 0.023. ^b 1.289 ± 0.014.

Standard deviations as shown were obtained by analysis of 20 subsamples.

and current for generation of exciting x-rays were found to be essentially the highest attainable with the instrument. Somewhat lower than maximum voltage and current yield higher emission to scatter ratios, but lower the actual intensity for the zinc emission maxima. This latter factor becomes important when analysis of small quantities of the element is involved. Throughout this investigation the instrument was operated at 50 kv. and 48 ma. A wave length discriminating reverter set to yield approximately 70% of maximum intensity at the zinc wave length was used in measurement of both emission and scatter as a means of discriminating against scatter. The wave length at which scatter radiation was measured was chosen because it is close to the wave length of zinc emission and it occurs at a point free of any possible interfering elemental emission maximum. The angle at which the scatter radiation is measured is empirical, but in this type of analysis it must be as close as possible to the angle where the zinc wave length is measured and yet far enough removed so as not to be affected by the zinc radiation. The first order zinc K_{α} maximum was measured at 41.80° 2 theta and the scatter radiation at 41.00° 2 theta.

Preparation of Samples and Counting Technique. Samples of dried plant material were ground to pass a 20-mesh stainless steel screen in a Wiley mill. Standards for reference were obtained by chemical analysis of reference samples using the Zincon method (77) and by addition of known amounts of zinc to reference samples. When zinc was added, the following procedure was used.

Sufficient acetone was added to 10 grams of dried, ground plant tissue (containing an unknown quantity of zinc) in a Mullite mortar just to wet the entire mass without excess. Aliquots

Table II. Effect of Addition of Zinc to Samples of Unknown Zinc Content

Tissue	Unknown		Unknown + 40γ Zn/G.		ΔF - S, c.p.s.	ΔF/S
	F - S, c.p.s.	F/S	F - S, c.p.s.	F/S		
Apricot	3.9	1.09	32.0	1.73	28.1	0.64
Prune	8.2	1.17	40.8	1.82	32.6	0.65
Almond	16.7	1.36	45.9	2.00	29.2	0.64
Apple	89.9	2.51	128.8	3.17	39.9	0.66
Apple	59.5	2.01	97.4	2.66	37.9	0.65
Apple	18.2	1.33	55.1	1.99	36.9	0.66
Quince	38.7	1.60	78.0	2.25	39.3	0.65
					Mean	0.65 ± <0.01 ^a
Corn	9.9	1.17	41.8	1.72	31.9	0.55
	21.3	1.37	46.1	1.90	24.8	0.53
	23.2	1.47	48.2	1.98	25.0	0.51
	12.4	1.26	41.0	1.82	28.6	0.56
	1.0	1.02	33.9	1.57	32.9	0.55
					Mean	0.54 ± 0.02 ^a
Milo	11.7	1.25	36.9	1.79	25.2	0.54
	8.2	1.18	37.2	1.72	29.0	0.54
	6.8	1.14	32.5	1.66	25.7	0.52
	15.3	1.32	36.9	1.82	21.6	0.50
	17.6	1.36	41.0	1.85	23.4	0.49
					Mean	0.52 ± 0.02 ^a
Tomato	5.7	1.11	31.6	1.69	25.9	0.58
	9.4	1.20	34.7	1.75	25.3	0.55
	9.9	1.22	32.6	1.74	22.7	0.52
	9.4	1.18	37.5	1.78	28.1	0.60
	8.7	1.19	33.7	1.75	25.0	0.56
					Mean	0.56 ± 0.03 ^a

^a Standard deviation of ΔF/S values.

of zinc sulfate solution were added to give the desired, added concentrations of zinc. After thorough mixing, the samples were placed in a 60° C. oven, until excess acetone and moisture were removed, and then remixed thoroughly in the dry state.

During analysis of samples, radiation intensity was measured in terms of time required for registration of 10,000 counts in order to stay within a standard deviation of 1% for a given intensity measurement (8). These values were converted to counts per second. Emission intensity values, *F*, as reported, represent the intensity of zinc emission plus scatter at 41.80° 2 theta.

A preliminary investigation of the effect of briquetting samples of fruit tree foliage under a pressure of 14,000 p.s.i. as contrasted to packing the samples by hand in a Bakelite sample holder of 3-mm. depth with a stainless steel spatula is summarized in Table I. Briquetting increased the precision of analyses, but because the precision obtained with loose-packed samples was sufficient for present purposes, subsequent data were obtained on loose-packed samples. The thickness of the samples was sufficient to mask any scatter from the holder.

Expression of Results and Calculations. For the purpose of calculation, the intensity of the scatter at the 2 theta angle where the zinc maximum is detected must be known. This was accomplished by measuring the scatter intensity at 41.80° 2 theta and at 41.00° 2 theta from materials known to be free of zinc. Their ratio established the

intercept for zero zinc concentration and also provided a constant multiplier for the calculation of the actual emission above scatter intensity. The ratio for pure calcium carbonate, silicic acid, starch, and filter paper was found to be a consistent value of 0.91. This value was used in the subsequent calculations.

In analyses involving the method of addition of known quantities of zinc, the initial concentration of zinc was calculated in one of two ways:

$$X = \frac{Y[(F/S)\mu - 0.91]}{(F/S)y - (F/S)\mu} \quad (1)$$

or

$$X = \frac{Y(F - 0.91S)\mu}{(F - 0.91S)y - (F - 0.91)\mu} \quad (2)$$

where *X* = unknown zinc concentration in the plant sample

Y = zinc added, γ/gram

F = radiation intensity, c.p.s. at 41.80° 2 theta

S = radiation intensity, c.p.s. at 41.00° 2 theta

μ = unknown sample

y = unknown sample plus *Y*

0.91 = *F*/*S* at zero zinc concentration

Equation 1 utilizes the emission to scatter ratio, whereas Equation 2 utilizes the increase in emission intensity effected by the known quantity of zinc added.

Results and Discussion

Consistent with the observation of Brandt and Lazar (2), a plot of emission intensity (*F*-*S*) as a function of concentration of an element in different samples gives a linear relationship (with a correlation coefficient, *r*, of

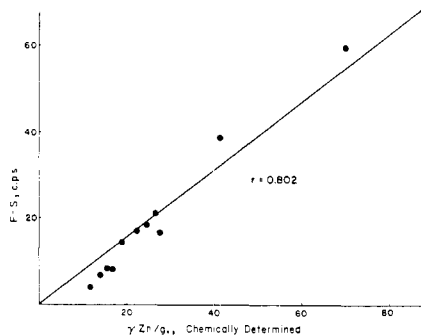


Figure 1. Emission intensity as a function of zinc concentration in deciduous tree foliage

0.802) as exemplified by the analysis of deciduous fruit tree foliage (Figure 1). When these same data are plotted using the ratio of intensity of zinc emission to scatter (F/S) as a function of concentration, a linear relationship with much better precision ($r = 0.999$) is obtained (Figure 2). This supports the conclusion that interferences within different samples are variable and that the interferences affect both the zinc radiation and the scatter radiation in similar manner within the range of concentrations encountered in this study. Similar plots of data in which increments of zinc were added to tissue samples established the validity of plotting emission to scatter ratio as a function of concentration within a particular sample.

A linear relationship between ratio and zinc concentration is not necessarily maintained between tissue of different species of plant. Table II shows the effects of addition of 40 γ of zinc per gram of tissue to a number of plant species. Some very significant relationships are noted in these data. Addition of the zinc to the deciduous fruit tree foliage resulted in an increase in the intensity of zinc emission above scatter of from 28.1 to 39.9 c.p.s. In addition, scatter intensity varied considerably from sample to sample. In spite of variations in emission and scatter intensities, however, the increase in emission to scatter ratios ($\Delta F/S$) remained nearly constant (between 0.64 and 0.66). This represents a variability of only ± 0.6 γ of zinc per gram of tissue at this level. The constancy of ratio increase with added zinc indicates that zinc emission is affected in the same way as the scatter within this group of similar species. The data also indicate that interferences are extremely variable within this class of materials. Figure 2, which shows the relationship between F/S and zinc concentration in various deciduous fruit tree species, as determined chemically, shows also a $\Delta F/S$ of 0.64 per 40 γ of zinc per gram of tissue.

Variability in emission to scatter ratio as a function of zinc concentration

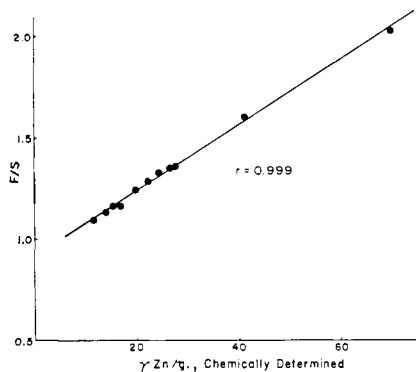


Figure 2. Emission to scatter ratio as a function of zinc concentration in deciduous tree foliage

between classes of plant materials is illustrated by results obtained with cereal, vegetable, and deciduous fruit tree species. Milo, corn, and tomato foliage responded to the addition of zinc in similar order of magnitude, but the response was different than that exhibited by deciduous fruit tree species.

Despite relatively large differences in intensity of zinc emission due to the addition of 40 γ of zinc per gram of tissue, actual intensity above scatter ($F-S$) can be used to calculate the zinc content of the original material using Equation 2. This technique would yield precise results in that interferences would be minimized, because the unknown samples and unknown plus zinc differ in composition only in the zinc content and the small amount of sulfur and oxygen added as sulfate. However, this does require the addition of zinc to every sample, a procedure which, as shown above, may not be necessary when dealing with similar types of plant material, especially where routine assay of a particular plant species is involved.

The results obtained with tissues of different species support the contention that factors other than absorption are operative in analysis of plant materials. Lower emission to scatter ratios obtained with corn samples as compared to fruit tree foliage may be due to variations in scatter level between the two classes of material. The data obtained do not define the reason for the difference noted, however.

Application of the x-ray emission method of analysis of zinc in a number of plant tissue samples is illustrated in Table III. Results obtained by both procedures outlined above agree well with results by chemical analysis.

Conclusions

Scatter radiation can be used as an internal standard in zinc analyses of plant materials. Reasonable accuracy in determination of zinc in plant samples can be attained from a standard curve, or by calculation, from data obtained

Table III. Comparison of Zinc Concentration in Deciduous Tree Foliage as Determined by Chemical Analysis and by X-Ray Emission Methods

Chemical Method, γ/g .	X-Ray Emission by Addition	
	F/S^a , γ/g .	$F-S^b$, γ/g .
11.6	11.9	11.1
98.4	100	94.4
69.8	68.3	68.6
18.9	19.4	19.1
24.4	25.5	25.1
22.2	22.8	22.1
26.5	26.3	27.2
41.2	41.8	45.1
13.9	13.1	13.9
16.8	14.9	15.1
15.5	15.2	15.7
27.5	27.3	28.6

^a Calculated by Equation 1.

^b Calculated by Equation 2.

by correlating ratio of zinc emission to scatter with zinc concentration in a particular kind of plant material. Such a relationship obtained with one kind of material cannot necessarily be applied to another. For high accuracy, a known quantity of zinc should be added in any case and the amount of zinc in a sample calculated either on the basis of emission to scatter ratio increase or actual emission intensity increase per unit of zinc added.

Results obtained in zinc determination may readily apply to determination of other elemental constituents of plants.

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