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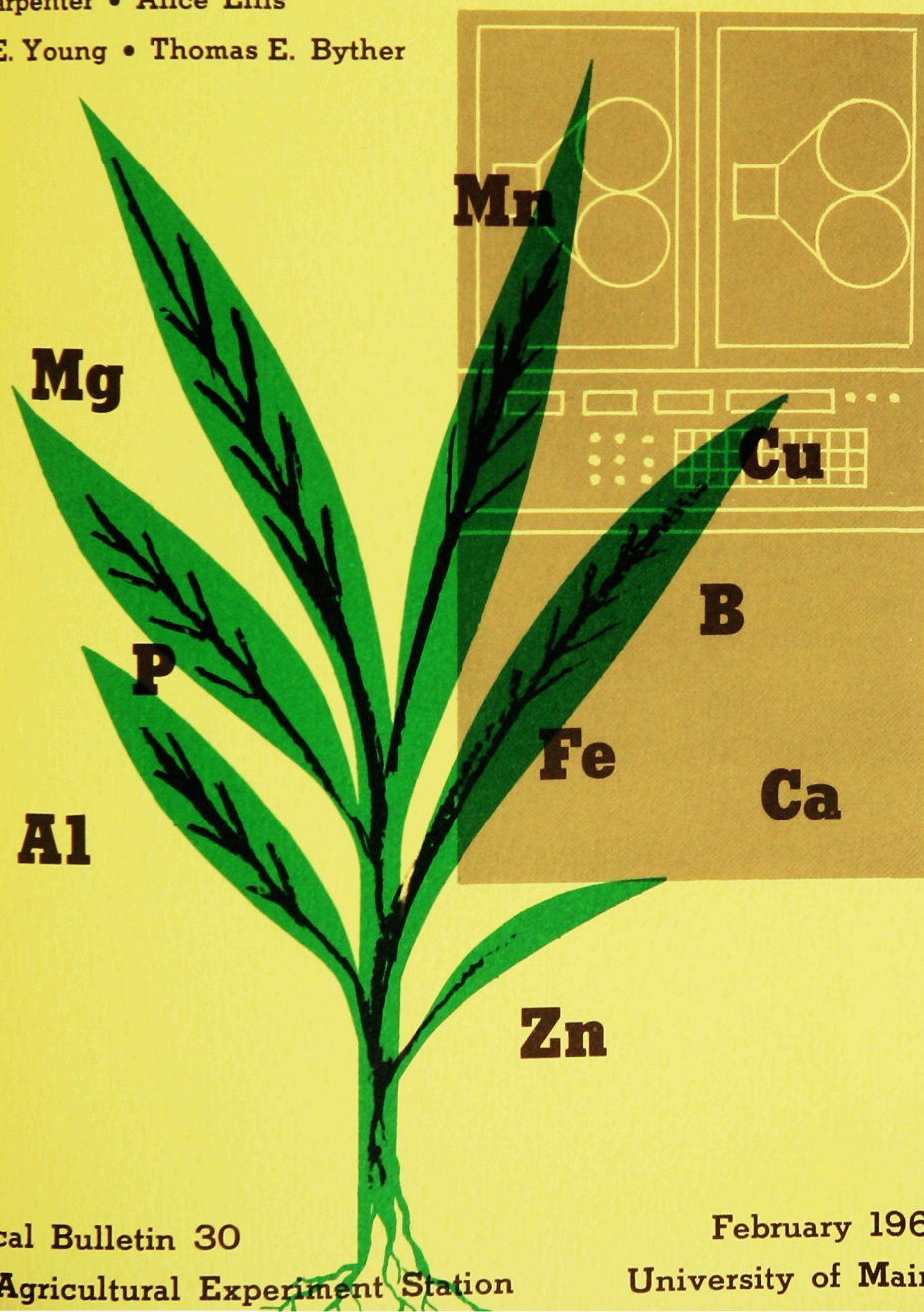
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A CRITICAL EVALUATION OF RESULTS FROM SPECTROGRAPHIC ANALYSIS OF PLANT TISSUE

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Paul N. Carpenter, Alice Ellis, Harold E. Young
and Thomas E. Byther¹

Spectrographic analysis of plant tissue for eleven elements, all at one time, is a relatively new technique and, as such, is subject to evaluation to determine its accuracy. In fact, publication of analytical results having to do with many of the minor elements has been meager. Some sources of information on elemental content are listed (1, 3, 4, 5, 7).

For this study, samples of wheat, corn, timothy, orchardgrass, alfalfa, Bermuda grass and tomato were analyzed for eleven elements.

A statistical study of these data was undertaken to determine the precision of the Maine Agricultural Experiment Station's spectrographic analyses and to determine the precision that could be expected from analyses by this method. This paper is a report of that study.

Four, 2 gram subsamples of each of seven samples were weighed into crucibles and ashed by muffle furnace at 550° C. for eight hours. The dry ash was transferred to polyethylene containers and 10 ml of a solvent, buffer, internal standard mixture consisting of a 20% solution of HCl to which was added CoCl₂ to produce 250 ppm. Co, and LiCl₂ to produce 5,000 ppm. Li. This solution was transferred to a boat as needed and injected into the arc by means of the rotating disc technique. Each of these subsamples was analyzed by spectrograph three times, making a total of 12 analyses of each sample for aluminum, manganese, molybdenum, calcium, phosphorus, magnesium, zinc, copper, iron, boron and potassium.

Instrumentation

The Baird-Atomic, 3 meter RDRS was used, with the following parameters:

Source:

AC spark; 7½ Amps., R.F. — 10 breaks/half cycle

Capacitance: .0025μf

Inductance: 40μh

Spark gap: 4 mm

¹ Associate Agronomist, Technical Assistant, Professor of Forestry, and Programmer, University Computing Center, respectively.

Entrance slit: 75 microns

Pre-burn: 10 sec.; Burn: 30 sec.

Spectrum lines and slit widths:

Cu — 3247.5 x 2	150 m	Fe — 2599.9 x 2	50 m
Ca — 3179.3 x 2	100 m	P — 2535.6 x 2	100 m
Al — 3092.8 x 2	100 m	B — 2497.7 x 2	25 m
Mn — 2949.2 x 2	100 m	Zn — 2138.0 x 2	100 m
Mg — 2790.2 x 2	100 m	K — 4044.1 x 1	100 m
Mo — 5533.0 x 1	100 m	Co — 2286.1 x 2	Internal standard

Synthetic standards and referee standards burned before and/or during each series of samples were used to recalibrate standard curves.

Statistical Analyses of Results

The results produced by spectrographic analyses were taken to the University Computer Center for statistical analyses for means, standard deviations, standard errors and coefficients of variation.

Analysis of variance was made with the F test and Duncan's Multiple Range Test for variability and Bartlett's Chi-Square Test for homogeneity. A summarization of these results is shown in tables 1, 2 and 3. Table 1 shows results of statistical analyses of seven species for eleven elements. Of these, 74 were suitable for statistical analysis and of these 67 showed no significant variation according to the F test. In only seven cases out of 74 were there significant variations. Of these seven cases, three were significant at the 5% level and four at the 1% level. Aluminum and iron each had two cases and calcium, copper and boron had one.

When the data were tested further by Duncan's Multiple Range Test, 53 cases out of 74 showed no significant variation between means. By this test, aluminum had six cases that were significantly variable; manganese three; calcium, copper, iron and potassium, two each; phosphorus and boron, one each; and magnesium, zinc and molybdenum had none.

From Bartlett's Chi-Square Test of the homogeneity of observations making up each mean, it was found that only four of 74 were significantly variable. There were two in manganese, one in boron and one in iron. These results would indicate the greatest sources of error were in analyses for aluminum, iron and manganese; with high confidence in results for magnesium, zinc, molybdenum, phosphorus and boron; and only slightly less confidence in results for potassium, calcium and copper.

When the means, standard deviations, standard errors and coefficients of variation were computed (table 2), standard deviations and coefficients of variation seemed about normal. The C.V.'s ranged from a low of 1.58% to a high of 41.68%. When these were arranged by classes it was found that there were 15 cases with C.V.'s under 5%, 27 between 5% and 10%, 20 between 10% and 15%, and 15 over 15%. The class with C.V.'s greater than 15% was disturbing. When these values were examined more closely it was noted that eight of the 15 occurred in analyses for three elements: manganese, molybdenum and copper. This left only one case, boron, that was not included in the above groups.

From these data (tables 1 and 2), it was found that the accuracy for manganese analyses was less than for the other elements, and copper analyses also showed a tendency to vary. Accuracy in molybdenum analyses may perhaps be more difficult because of the low level of concentration of the element in most samples.

A more important point is that several cases showed abnormally high C.V.'s as compared to the group as a whole. It was judged that if a C.V. was more than twice the average of those for that element, it would be classed as abnormal. When this was done, there appeared to be nine cases which fell in this category with eight of the nine occurring in two samples (samples 5 and 6). These cases were: sample 1, copper, 31.53%; sample 5, manganese, 39.56%, phosphorus, 18.41%, zinc, 21.81%, iron, 33.83%, and potassium, 16.26%; and sample 6 with magnesium, 12.13%, copper, 40.68%, and boron, 41.65%.

There was no consistency among elements in this respect, thus eliminating the possibility that these were analytical characteristics pertaining to one or more elements. However, since these two samples (5 and 6) contained most of the results classed as abnormal, it was suggested that the original samples were not homogenous. If they had been homogenous, analytical results would have paralleled those of the other samples, and if they had been elemental analytical errors, they would have appeared in the same elemental results. Since these did not occur it would appear that a part, if not most of the variation which appeared in these results was caused by something other than the analytical process.

In order to check samples 5 and 6 to determine if the exceptionally large errors in these samples were due to poor mixing or particle size separation, the samples were reground through a finer screen on the Wiley mill and mixed. There was sufficient sample material to re-anal-

alyzed samples 1, 5, 6 and 7. When this was done, the following changes in C.V. occurred: sample 1 for copper changed from 31.53 to 10.78; sample 5, Mn, changed from 39.56 to 22.01, P from 18.41 to 6.07, Zn from 21.81 to 8.17, and Fe from 33.83 to 10.07; and for sample 6, Mg from 12.13 to 6.29, Cu from 40.68 to 39.24, and B from 41.65 to 25.35. All other C.V.'s remained quite constant except, as a whole, all C.V.'s were decreased slightly.

These new C.V.'s are much more consistent with the general run of C.V.'s for each of the elements. These data then suggested that in sample 5 separation by particle size and regrinding eliminated most of this error. Sample 6 did not respond as well since the Cu and B C.V.'s were still excessively large. The cause of these large variations remains unknown.

If we now examine these results substituting the C.V.'s found after regrinding (table 2) for these abnormal C.V.'s, we find only 10 cases in which C.V.'s were greater than 15%. Three of these occurred each in manganese and copper, two in molybdenum and one each in boron and potassium. Using C.V.'s as a criterion of precision it would appear that the elements should be listed in the following order: calcium at about 5%; magnesium, phosphorus, aluminum, potassium, zinc, boron, molybdenum, iron, manganese and copper at about 15%.

In summary, these data indicate a greater precision of analysis than was expected. They also indicate areas of concern, namely, copper and manganese analyses, as indicated by C.V.'s and manganese by Bartlett's Chi-Square Test. Concentrated study of these areas might improve results. A second point of concern appeared with the excessively large variations in results from samples 5 and 6.

The elemental analysis is usually the last procedure accomplished in this type of work. When errors occur, the source of these errors is considered to be the last procedure before the errors appear, in this case, the spectrographic analysis. These results indicate the probability that these errors are not occurring at this point, but rather at an earlier stage in the sampling procedure. Baker et.al (2) lists many of the pitfalls in analysis of plant material for minor elements. This study indicates that lack of sample homogeneity may contribute a large part of the errors that appear in plant sample analysis.

Personnel of this laboratory have observed many cases of sample preparation which have led to analytical error. Among them are sample contamination—due either to improper handling of samples or inadequate cleaning. Sample contamination when analyzing for the major

elements was rarely observed, but with the minor elements, care must be exercised to remove dusts, either from soil or from fungicide-insecticide coverage, and also to prevent addition of contaminating elements by contact with contaminating containers or sampling implements.

A second, and common source of analytical error has been improper pulverizing (grinding) of samples. Plant samples such as leaf tissue may contain two or more types of material which, upon grinding, produces particles of two or more sizes and densities. Plant leaves may contain rough, wood-like material at the midrib and fine powdery material in the intervein areas. The woody material will grind only as fine as the screen size used, but the powdery material may be a much finer size particle.

After standing for even a short time the various size particles may separate, leaving a sample stratified by particle sizes, which is extremely difficult to re-mix into a homogenous sample. One solution to this problem is to grind the whole sample to the size of the finer particles. This method of regrinding was used with samples in this study and seemed to solve the problem with sample 5 but did not wholly take care of sample 6.

Table 3 lists the statistically estimated number of replicates necessary to produce results within the listed degree of error based on these data. From these data it was apparent that by using only five replicates an accuracy within 10% could be achieved at odds of 9 to 1 for the elements Al, p, Ca, Mg, Zn, and Fe. However, for the other elements up to 10 replications would be needed to achieve the same accuracy. It is, of course, true that at additional cost and time expenditure, additional replications would result in greater accuracy.

Should there be a need of accuracy of results better than 9 to 1, such as 19 to 1 odds, then more replicates would be needed. The average number of replicates would then be 7.6 with a low of 4.7 and a high of 10.4. Should less accuracy be acceptable, such as within 20% of the mean, then it would generally be acceptable to use only three replicates.

Discussion

Because the development of new techniques is always subject to evaluation by its users, it was of value to study the results from spectrographic analysis of plant tissue to determine the level of confidence that could be expected by its use.

Past experience has shown the degree of confidence that could be

expected through chemical analysis by the conventional methods of various materials for many elements. In many cases, this degree of confidence is very high, with errors expected to be in the order of .5% to 2.00%. Analyses of other elements and/or materials have produced errors at a higher level when analyzed by conventional methods.

Spectrographic analyses have produced errors generally higher than the best analyses for the common or major elements, i.e., greater than 2%. Although it has generally been thought that spectrograph errors were larger than errors by conventional methods, little has been published on the subject. This study has recorded spectroscopic data in order to establish some guide lines in predicting the accuracy that could be expected by these methods and so determine whether resulting data would be satisfactory for the purpose for which they would be used. These results seem to justify rather high confidence in the results of analysis for most of the elements examined. Those elements indicating results of lower degree of confidence should stimulate study to determine adaptations of procedures whereby confidence in these results may be increased.

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#5												
Alfalfa	F values	2.541	2.667	1.914	.271	.344	.229	1.455	.909	4.957*	4.554*	.445
	Chi-square	2.582	10.104*	2.141	6.188	6.047	3.181	N	N	1.280	2.235	3.917
	Duncan's	S	S	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	S	S	N.S.
#6												
Bermuda grass	F values	13.409**	.045	1.926	4.640*	1.311	2.167	.858	25.586* [†]	.593	.297	.656
	Chi-square	N	4.438	1.960	.494	N	3.530	3.427	N	N	1.909	5.216
	Duncan's	S	N.S.	N.S.	S	N.S.	N.S.	N.S.	S	N.S.	N.S.	N.S.
#7												
Tomato	F values	.634	.277	— ^x	— ^x	.421	.153	— ^x	1.643	.648	.918	1.085
	Chi-square	2.240	.866	—	—	6.680	1.724	—	3.923	8.086*	10.802*	3.295
	Duncan's	N.S.	N.S.	—	—	N.S.	N.S.	—	N.S.	N.S.	N.S.	N.S.

[†] F .05=4.07
.01=7.59

*=(.05)
**=(.01)

[‡] Chi-square .05=7.81

^x The data on three sets of means were not acceptable for statistical analysis.

TABLE 2. Means, standard deviations and coefficients of variation.

Sample		PPM Al	PPM Mn	PPM Mo	% Ca	% P	% Mg	PPM Zn	PPM Cu	PPM Fe	% B	% K
#1												
Wheat	Mean	36.17	94.00	.9992	.1506	.1302	.0603	18.75	4.38	108.25	4.15	1.41
	S.D.	1.99	10.32	.0604	.0061	.0076	.0023	1.29	1.38	8.97	.58	.17
	C.V.	5.50	10.98	6.04	4.03	5.85	3.89	6.87	31.53	8.28	13.99	11.89
								(10.77) ^Σ				
#2												
Corn	Mean	53.67	39.58	3.83	.6153	.2851	.3708	32.58	6.00	268.75	6.76	1.42
	S.D.	4.33	6.83	.62	.0426	.0186	.0279	1.16	1.16	19.55	.80	.20
	C.V.	8.08	17.25	16.27	6.92	6.51	7.51	4.98	19.40	7.28	11.81	13.91
#3												
Timothy	Mean	115.42	29.42	4.01	.5785	.3521	.2875	74.25	9.10	282.08	8.58	4.15
	S.D.	8.94	3.44	.21	.0149	.0092	.0045	9.18	.94	29.01	1.03	.26
	C.V.	7.74	11.73	5.15	2.58	2.60	1.58	12.10	10.37	10.29	12.03	6.88
#4												
Orchard grass	Mean	42.33	50.75	2.27	.3429	.2689	.3592	32.75	8.96	157.33	4.14	5.00
	S.D.	3.50	7.05	.36	.0176	.0144	.0211	2.38	1.38	10.68	.79	.26
	C.V.	8.27	13.89	15.92	5.14	5.36	5.90	7.26	15.39	6.79	19.08	5.21

#5

Alfalfa	Mean	79.75	24.25	11.57	1.428	.2364	.2770	29.67	6.23	180.24	31.17	1.13
	S.D.	8.34	9.59	1.15	.092	.0435	.0206	6.47	.77	60.97	1.40	.18
	C.V.	10.45	39.56	9.99	6.43	18.41	7.43	21.81	12.33	33.83	4.50	16.26
		(22.21) ^Σ			(6.07) ^Σ			(8.17) ^Σ		(10.07) ^Σ		

#6

Bermuda grass	Mean	87.50	54.92	4.69	.5215	.3732	.3724	83.08	2.17	279.08	4.23	2.21
	S.D.	9.77	9.21	.42	.0479	.0158	.0452	8.46	.88	33.07	1.76	.28
	C.V.	11.16	16.77	8.93	9.18	4.24	12.13	10.18	40.68	11.85	41.65	12.65
					(6.29) ^Σ			(39.24) ^Σ		(25.35) ^Σ		

#7

Tomato	Mean	53.42	480.83	29.50	3.338	.3440	.7100	155.00	4.39	183.17	88.25	3.52
	S.D.	3.82	16.76	1.08	.149	.0203	.0316	9.13	4.4	14.67	3.17	.18
	C.V.	7.76	3.48	3.66	4.47	5.88	4.45	5.88	10.20	8.01	3.59	5.09

^Σ Repeated analysis after regrinding.

TABLE 3. Estimated number of observations per sample necessary to yield data within 10% of the mean at odds 9 to 1 and 19 to 1 under the same conditions of sample and analysis.

		Al	Mn	Mo	Ca	P	Mg	Zn	Cu	Fe	B	K
#1												
Wheat	9 to 1	3	6	4	3	3	3	4	29	5	8	6
	19 to 1	5	8	5	3	5	3	6	41	6	11	8
#2												
Corn	9 to 1	5	10	10	4	4	4	3	13	4	6	8
	19 to 1	6	14	13	6	6	7	4	17	7	8	10
#3												
Timothy	9 to 1	5	6	3	3	3	3	7	5	5	6	4
	19 to 1	8	8	4	3	3	3	9	7	7	9	6
#4												
Orchard grass	9 to 1	5	8	9	3	3	3	4	9	4	12	3
	19 to 1	6	10	13	4	5	5	7	12	6	17	5
#5												
Alfalfa	9 to 1	5	45	5	4	12	4	15	7	34	3	10
	19 to 1	7	63	7	6	16	7	21	9	47	4	13
#6												
Bermuda grass	9 to 1	6	10	5	5	3	7	5	47	6	49	7
	19 to 1	8	14	6	6	4	9	7	67	8	70	9
#7												
Tomato	9 to 1	4	3	3	3	3	3	3	5	5	3	3
	19 to 1	7	3	3	4	5	4	4	7	6	3	4