
SPECTROGRAPHIC TECHNIQUES AND ANALYSES OF PINE NEEDLES

J. D. SAYRE

*Crops Research Division, ARS, U. S. Dept. of Agriculture,
and the Ohio Agriculture Experiment Station, Wooster*

The spectrograph can be used to determine many elements in plants, although all elements cannot be determined equally well because of volatility, excitation, or ionization differences. Some 12 to 20 elements are rather easily determined in the open arc in plant ash in the procedure used here. They are boron, phosphorus, magnesium, lead, tin, silicon, iron, aluminum, molybdenum, calcium, vanadium, copper, silver, zinc, sodium, titanium, nickel, cobalt, wolfram, manganese, potassium, and chromium. Successful analyses can be made on very small samples on a spectrograph. Most samples are 100 milligrams in weight but smaller ones, down to 10 milligrams, can be used if necessary. This small sample size is an advantage when studying the margins and tips of leaves, especially the tips of pine needles.

In brief the spectrographic procedure consists of burning the samples in an open arc and recording the spectrum from this arc which contains the unknowns on a photographic plate. The spectrograms when properly developed and fixed constitute a permanent record of the elements in the samples. The light or radiation from the arc is dispersed through a quartz prism and each element shows up on the plate as a line of specific wave length or a group of lines characteristic of the element. The photographic emulsion also integrates the light energy from the elements and

forms a dense line when a quantity of the elements is present and a very faint line when only a small amount is present. This is the basis of the quantitative nature of the procedure.

The spectra of the elements on the plates are fine lines and must be examined and measured under a magnification of about 10 to 20 diameters for best results. Hence, a microdensitometer is a necessary part of the spectrographic laboratory.

METHODS

Location of lines.—The recognition, location, and determination of the origin of spectral lines could be a long job. But it is possible to locate and recognize those lines of the elements used in quantitative analysis in a few days time. The following is a list of those lines most commonly used to determine the given elements.

<i>Element</i>	<i>Angstrom</i>	<i>Element</i>	<i>Angstrom</i>
Boron	2497.7	Copper	3274.0
Phosphorus	2535.7	Silver	3280.0
Magnesium	2779.9	Zinc	3282.3
Lead	2833.1	Sodium	3302.3
Tin	2840.0	Titanium	3349.0
Silicon	2881.6	Nickel	3414.8
Iron	3020.6	Cobalt	3453.5
Aluminum	3092.7	Wolfram	4008.8
Molybdenum	3170.3	Manganese	4030.8
Calcium	3179.3	Potassium	4044.1
Vanadium	3184.0	Chromium	4254.3

The spectral lines are located (1) by means of an angstrom scale on the spectrograph, (2) by means of a reference spectrum, usually iron on high dispersion instruments or mercury on quartz prism spectrographs, or (3) by the characteristic pattern of lines for each element. Unconsciously all three methods may be used even though the angstrom scale or a reference spectra may not be consulted, or the pattern found. For with very little practice, the cyanogen bands or other known lines or groups of lines serve as a reference point and in a short time a dozen elements can be quickly recognized and measured.

Spectrographic arc.—The theory and construction of spectrographic sources is an involved and complicated study, but the practical use of such an arc is as simple as turning on and off an electric light. Flat top purified graphite electrodes are used to support the samples, and the arc or spark is maintained between them in the proper position in front of the spectrograph.

Plate calibration.—The photographic emulsion is not an ideal integrator of light energy. It may vary with the wavelength of light and not show a true linear response with intensity. It must be calibrated to show the amount of light necessary to produce a given blackening of the emulsion. This is an involved process and requires step sector discs, constant light source, special graph paper (Seidel paper), and controlled development of the plate. The end result is a table showing the transmission of the spectral lines and the intensity of light necessary to produce them. So all one has to do is read a table.

Working curves.—Working curves are constructed by plotting intensities from known amounts of the elements being studied. They are similar to working curves of substances determined in colorimetric analysis. They are made from synthetic mixtures of salts similar in composition to plant ash, with added known amounts of the required elements to be studied. Some are very easy to construct, but others, where enhancement or suppression of lines results from the presence of certain other elements or ions in the mixture, are very difficult to obtain.

TABLE 1

Boron content of red pine needle samples, from E. L. Stone, Professor Forest Soils, Cornell University. Plot B-12, 22 year old, sampled October 22, 1953 from mid node position, height 22 feet

Sample No.	Sample Data				Spectrographic				Boron		
	Year growth	Needle tips cm	Dry wt. gm	Per cent ash	Boron 2798		Lithium 2741		Ratio B/Li	μg in 10 mg ash	μg in 100 mg tissue
					T	G	T	G			
117	1953	0-1	.8	2.23	17	188	12.5	258	.73	29	6.5
					24	129	13	248	.52	18	4.0
118	1953	1-2	.7	1.52	30	100	10	327	.31	10.4	1.58
					28	108	9.2	356	.30	10	1.52
119	1953	2-	10.4	1.66	39	72.2	9	365	.20	6.5	1.08
					40	70.0	9.4	348	.20	6.5	1.08
120	1952	0-1	.9	3.02	12	269	9.8	333	.81	34	10.3
					16	200	10	327	.61	22	6.7
121	1952	1-2	1.0	2.03	41	67.9	10	327	.21	6.7	1.36
					35	83.0	8.5	393	.21	6.7	1.36
122	1952	2-	13.9	1.92	56	42.0	10	327	.13	3.4	.65
					53	45.8	9	365	.13	3.4	.65

No. = Original sample number, from E. L. Stone.
 Cm = Part of needle in each sample, from tip down.
 T = Transmission in percent.
 G. = Gamma value or relative intensity.
 Spectrographic Plate No. 26, Book No. 11, by David Chou.

TABLE 2

Elements in red pine needle samples

* No.	P 2536 G	Mg 2802 G	Pb 2833 G	Si 2882 G	Fe 3020 G	Al 3092 G	Ca 3179 G	Cu 3274 G	Ag 3280 G	Mn 4035 G	K 4044 G	Zn 4810 G	Li 2741 G
117	53	158	62	269	142	129	77	177	41	100	15	25	258
	47	136	66	248	136	123	66	167	43	86	14	26	248
118	46	149	62	80	50	77	86	230	49	72	24	19	327
	50	149	68	83	50	80	93	230	52	68	24	18	356
119	48	142	55	70	230	60	66	89	49	64	29	15	365
	42	136	62	66	113	53	66	96	53	53	25	14	348
120	53	89	43	258	129	136	108	136	33	177	9	14	333
	37	83	68	230	129	129	93	136	37	177	8	12	327
121	33	72	46	68	45	75	123	113	36	89	15	8	327
	37	75	50	68	49	80	123	123	37	89	20	6	393
122	37	113	83	93	50	70	89	80	36	96	18	9	327
	42	118	108	108	60	80	104	96	45	104	24	10	365

G = Gamma values or relative intensity.
 * = See table 1 for other data on same samples.

TABLE 3

Elements in white pine needle samples from E. L. Stone, Professor Forest Soils, Cornell University. Plot B-22, 19 year old, sampled October 31, 1953

Sample No.	1953 growth	Needle tips cm	B	Si	P	Mg	Mn	Pb	Fe	Al	Ca	Cu	Ag	Zn	K	Li
			2498 G	2507 G	2536 G	2779 G	2801 G	2833 G	3020 G	3093 G	3159 G	3274 G	3280 G	3282 G	4044 G	2741 G
236	Apical 2 cm	0-.5	117	167	28	32	108	22	83	356	20	43	19	13	8	53
			142	158	24	31	104	20	80	333	18	43	18	12	9	52
237	Apical 2 cm	.5-1.0	75	72	24	37	100	21	100	200	24	47	16	14	10	53
			62	62	22	37	93	22	89	182	22	46	17	12	10	55
238	Apical 2 cm	1.0-	28	20	30	59	72	22	38	142	22	22	13	12	12	57
			22	17	24	52	64	29	34	129	19	25	14	12	12	62
239	Basal 5 cm	0-.5	50	149	26	31	93	20	89	356	18	47	14	10	7	53
			39	136	24	31	96	21	104	327	17	52	14	10	7	57
240	Basal 5 cm	.5-1.0	31	41	24	32	93	18	46	158	22	43	14	11	11	64
			24	35	18	29	83	17	42	158	20	39	14	10	12	64
241	Basal 5 cm	1.0-	22	28	30	44	66	17	43	123	20	22	12	11	12	57
			15	22	22	39	60	20	36	118	18	27	11	12	12	62

G = Gamma value or relative intensity.

Spectrographic Plate No. 27, Book No. 11, David Chou.

TABLE 4

White pine needles, near greenhouse, Ohio Agr. Expt. Station May, 1955

	B 2798 G	Si 2881 G	Fe 3020 G	Al 3093 G	Mn 4035 G	Mg 2802 G	Li 2741 G	
Tip 1	108	136	104	248	96	80	72	
2	24	41	64	86	83	86	75	
3	10	50	68	104	68	96	104	
Base 4	5	35	46	70	35	64	104	
	P 2536 G	Pb 2833 G	Ca 3178 G	Cu 3274 G	Na 3302 G	Ag 3280 G	Zn 3282 G	K 4044 G
Tip 1	14	26	12	37	12	7	6	32
2	8	30	18	39	16	7	5	41
3	11	41	32	53	20	12	8	64
Base 4	8	33	35	53	22	12	8	66

Spectrographic Plate No. 52, Book No. 11, A. R. Correll.

Accuracy and expression of results.—Spectrographic analyses can be very accurate for certain elements, especially when these are present in very small amounts. The National Bureau of Standards accepts the spectrographic results over chemical results in many instances where only small amounts are involved. In most of the work reported in this paper only large differences in concentration are discussed.

The results of spectrographic analysis can be expressed just as any other analysis, in percent or parts per million, but sometimes this is very difficult because

of the very small amounts involved. It is often better to express the results in comparative amounts rather than in absolute values. One should know the copper constant of an ore very accurately; but since all biological material contains a little copper, it is often more important to know the relative amount of copper in one tissue as compared to another than to have the absolute amount in each. And it is much easier to do this.

Starting with the spectra of a series of plant samples on a spectrographic plate, one can first of all visually examine the density of the lines in the different spectra and see whether there is more in one than in another. Next the lines can be measured with the densitometer and compared. The values of the density measurements can be corrected for emulsion response, and for spectrogram variations (internal standard), and further compared. The values can be expressed in absolute amounts in the sample mixture from prepared working curves. Finally, if the ash content of the dried tissue is known, they can be expressed in amount per unit of dry tissue and if moisture were determined on the wet plant tissue, then in amounts in the wet tissue.

RESULTS

This work, resulting from a rather extensive program on the study of mineral elements in the margins and tips of corn leaves, has been extended to many other plants such as sorghum, sugarcane, wheat, oats, grass, iris, lily, wheat awns, and pine needles.

Only the results on pine needles are discussed here. The samples were obtained from New York State through the kindness of E. L. Stone at Cornell and from the campus and arboretum here. Several graduate students and technicians besides myself have worked on the samples.

The results of the boron analyses in the first set of samples from New York State are tabulated in table 1 in some detail to show how it is done. These data show the high accumulation of boron in the tips of needles.

Table 2 shows the results of spectrographic examination of the same set of samples for eleven other elements besides boron. These data are summarized as gamma values or relative intensities only to save space. They show that, in addition to boron, silicon, manganese, iron, copper, and aluminum are higher in the tips than in the rest of the needle. Phosphorus, magnesium, lead, calcium, silver, or zinc, show no difference; potassium is slightly lower in the tips than in the other parts of the tissue.

The data in table 3 show the elements examined in white pine needles. The sample size was too small for a 90-10 buffer mixture, so a mixture of 100 milligrams of LiF and 100 milligrams of tissue were used. The results are recorded in relative intensities and they show a high accumulation of boron, silicon, aluminum, manganese, iron, and copper in the tips and about equal amounts of the other elements throughout the needle, with the exception of potassium which is lower in the tips.

A set of white pine needle samples was obtained by Tzu-liang Yuan at Wooster and separation or dissection made into 4 parts. These samples were put on Plate No. 52 by A. R. Correll and the results are shown in table 4. Here again there was an accumulation of boron, silicon, aluminum, iron, and manganese in the tips of the needles, with six of the elements about equally distributed in the needle, and potassium again lower in the tips than elsewhere.

SUMMARY

It appears from these studies on pine needles that boron, silicon, manganese, aluminum, iron, and copper accumulate in the tips; that phosphorus, magnesium, lead, calcium, silver, and zinc are uniformly distributed and that potassium is lower in the tips and high in the base of the needle.