Instrumental Determination of Trace Elements in Plant Tissue by Neutron Activation Analysis and Ge(Li) Gamma-Ray Spectrometry

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By use of neutron activation and a large-volume high-resolution Ge(Li) detector the practicality of determining the concentrations in plant tissue of 15 elements—Mn, K, Cu, Na, As, Br, P, Rb, Cr, Fe, Hg, Se, Zn, Cs, and Co—was demonstrated. This instrumental method, which requires no chemical or ashing manipulation, eliminates many inherent errors associated with chemical determinations. The samples are freeze-dried, neutron-activated, and analyzed by Ge(Li) gamma-ray spectrometry. The resulting spectra are reduced by standard techniques in which the corrected counts are compared to a system of standards to obtain elemental concentrations.

Several studies of the effect of trace element concentrations on plant growth have been performed (Kuhn *et al.*, 1961; Monier and Williams, 1949); however, at present the trace elements essential to plant growth have been only partially defined (Lamb *et al.*, 1957). As the sensitivity and selectivity of analytical and bioassay techniques are increased, the list of essential trace elements will grow. In addition to the need for essential elements, for some elements there is a certain critical concentration range for healthy plant development (Lamb *et al.*, 1957). There are also many interrelated functions associated with trace element concentrations (Bowen, 1966; Mitsui *et al.*, 1962).

A major limitation to extensive trace element studies in plant samples has been the lack of practical methods for the measurement of a large group of trace elements in the parts-per-million to parts-per-billion range. Methods of analysis vary from time-consuming wet chemical methods for the individual elements (Horwitz, 1955) to spectrographic and flame photometry procedures where several elements may be determined instrumentally (Mathis, 1954; Tipton *et al.*, 1963; Wenner, 1958). Bedrosian *et al.* (1968) indicate that a direct multielement determination by spectrographic analysis is feasible. However, in general the multielement procedures lack selectivity and sensitivity for measuring many trace elements of interest, and they require a wet- or dry-ashing step where the more volatile elements such as Br, Se, and As could be lost. An even more serious problem is random contamination. Where lengthy chemical reduction, such as ashing, is necessary, contamination of samples is always a potential problem. In some cases the trace elements of interest in plant tissue are present in lower concentrations than in pure reagents used for chemical separation (Robertson, 1968).

Over the last decade neutron activation analysis has become an increasingly useful tool for trace elemental analysis. A chief advantage is sensitivity; for many elements, activation analysis is the most sensitive technique known. Another advantage is that reagent contamination of the sample can be avoided where no pre-irradiation chemical treatment is conducted. Several authors have described methods for multielement measurements in biological materials (Bowen *et al.*, 1963; Lyon, 1964; Wester, 1965) by neutron activation analysis. However, the large amount of radiochemistry required prior to gamma-ray spectrometry in these methods limits their wide application. A Ge(Li) γ-ray spectrometer could greatly extend the selectivity for activation analysis of biological tissue (Perkins *et al.*, 1967). The purpose of this study was to develop a reasonably simple and essentially contamination-free scheme for the multielement analysis of plant tissue samples by neutron activation and Ge(Li) spectrometry.

SAMPLE PREPARATION

The main emphasis in the study has been to establish method feasibility rather than to relate trace element concentrations to environmental or biological parameters. Several special techniques were developed and employed in sample handling and encapsulation prior to irradiation. Pre-irradiation sample preparation was performed in a clean air hood (Agnew Higgins Model 43) to minimize contamination from dust in the air. For samples of high moisture content, such as pears, internal portions were first sectioned out with a Lucite knife; then an inner section was taken with a Lucite borer ($^3/_8$-inch diameter by 1-inch length). These samples were placed in preweighed

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polyethylene containers (Nalgene No. 25) which had been cleaned by leaching for 10 minutes with doubly distilled H_2O, then 10 minutes with doubly distilled concentrated HNO_3. Grain samples such as beans were placed directly in the irradiation container. Their sample weight was obtained by weighing the containers before and after sample addition. The samples were dehydrated by drying for 2 days in a Thermovac freeze-drying unit; this eliminates the possible loss of volatile elements. Losses of mercury up to 15% in drying biological material at 110° C. have been reported (Wester, 1965). To avoid contamination from the air after the freeze-drying process, air entering the chamber was passed through a sintered-glass filter packed with a Gelman 10-mμ pore size filter (Gelman Instrument Co., Chelsea, Mich.). After freeze-drying, the polyethylene containers were taped shut with plastic tape and wrapped in several layers of 0.003-cm. aluminum foil prior to activation. The foil prevented direct contact of the polyethylene containers with the irradiation facility and minimized external contamination of the polyethylene.

STANDARDS

The high resolution of the spectrometer systems allowed the use of mixed standard solutions which contain known concentrations of all the elements of interest. Because of chemical incompatibility of some elements, three standard solutions are used.

NEUTRON IRRADIATION

The samples were irradiated to an integrated neutron exposure of 7×10^{17} neutron per sq. cm., as measured by Cu foils, in a graphite-moderated reactor. A set of standards which included elements with a large variation in infinite dilution resonance integrals for neutron capture were simultaneously irradiated in each reactor position used in these experiments. The ^{64}Cu and ^{124}Sb activities produced were constant within the counting statistics of $\pm 5\%$, demonstrating that both the thermal and epithermal fluxes across the irradiation positions were flat. Table I shows the decay characteristics of the neutron-induced radionuclides.

Table I. Decay Characteristics of Daughter Radionuclides Produced by Neutron Irradiation of Plant Tissue

Parent Element	Daughter Radionuclide	Half Life	γ-Rays Measured, M.E.V.	Other Prominent γ-Rays, M.E.V.
Mn	^{56}Mn	2.6 hours	0.845	1.81
K	^{42}K	12.5 hours	1.523	...
Cu	^{64}Cu	12.8 hours	0.511	...
Na	^{24}Na	15.0 hours	1.367	2.752
As	^{76}As	26.3 hours	0.559	...
Br	^{82}Br	35.8 hours	0.777	0.554, 0.618, 1.317
P	^{32}P	14.3 days	Bremsstrahlung	...
Rb	^{86}Rb	18.6 days	1.079	...
Cr	^{51}Cr	27.8 days	0.320	...
Fe	^{59}Fe	45.1 days	1.095	1.292
Hg	^{203}Hg	46.9 days	0.279	...
Se	^{75}Se	120.4 days	0.264	0.279, 0.139
Zn	^{65}Zn	245 days	1.115	0.511
Cs	^{134}Cs	2.05 years	0.797	0.605
Co	^{60}Co	5.2 years	1.172	1.332

POSTIRRADIATION TREATMENT

After irradiation the samples were allowed to decay for 12 hours. The outer aluminum wrap was then removed from the polyethylene container and the outer surfaces were heavily lacquered to prevent transfer of contamination during sample discharge. The cap of the polyethylene container was removed and the irradiated sample was transferred to a 1-inch dish for γ-ray analysis.

SOLID-STATE Ge(Li) SPECTROMETER

The solid-state Ge(Li) spectrometer was a 20-cc. coaxial drifted diode with less than 10% uncompensated material. By operation with a 1408A Canberra preamplifier, a Tennelec TC 200 amplifier, and a Nuclear Data 2200 multiparameter analyzer, a system resolution as measured at the ^{60}Co 1.33-m.e.v. line of 3.4 k.e.v. was obtained. The peak to Compton ratio for the 1.33-m.e.v. ^{60}Co γ-ray was 12 to 1.

DATA ACQUISITION

After opening, the samples and standards were placed in 1-inch diameter counting dishes, coated with a thin layer of rubber cement to avoid loss of material, then covered with a double layer of 0.5-mil plastic to avoid any possible contamination of the Ge(Li) diode counting facility. A 20-minute count on the Ge(Li) spectrometer was taken to measure the short-lived activation products, ^{24}Na, ^{42}K, ^{56}Mn, and ^{64}Cu. Following a decay period of about 48 hours, the samples were recounted to measure ^{76}As and ^{82}Br, then allowed to decay an additional 8 weeks to permit these rather short-lived radionuclides to decay. During this decay period the troublesome bremsstrahlung radiation, mainly due to ^{32}P energetic beta-particles, is reduced by about 16-fold. The samples were then counted for 1000 minutes to measure the long-lived activation products, ^{32}P, ^{51}Cr, ^{59}Fe, ^{60}Co, ^{65}Zn, ^{75}Se, ^{86}Rb, ^{134}Cs, and ^{203}Hg. The neutron-activated standards were counted at the appropriate times and compared directly with the samples. Figure 1 shows a gamma spectrum of samples recorded at various times after irradiation. The concentrations of elements in the sample were calculated by Equation 1,

$$X = (C_1/C_2)\,(W_1/W_2) \qquad (1)$$

where X = concentration of element, grams; C_1 = counts per minute of sample; C_2 = counts per minute of standard; W_1 = weight of element in standard, grams; and W_2 = weight of sample, grams.

Even with the high resolution obtainable with the Ge(Li) γ-ray spectrometer, some of the radionuclides are not defined uniquely by γ-ray energy. However, they may be identified and measured by standard nuclear techniques. The 280-k.e.v. photopeak used to measure to ^{203}Hg is actually composed of the 280-k.e.v. ^{75}Se γ-ray and a 279.3-k.e.v. ^{203}Hg γ-ray. The ^{75}Se content can be measured easily by its 265-k.e.v. γ-ray and the intensity ratio of the 265- and 280-k.e.v. γ rays can be determined from the spectrum of a pure ^{75}Se standard. The ^{203}Hg concentra-

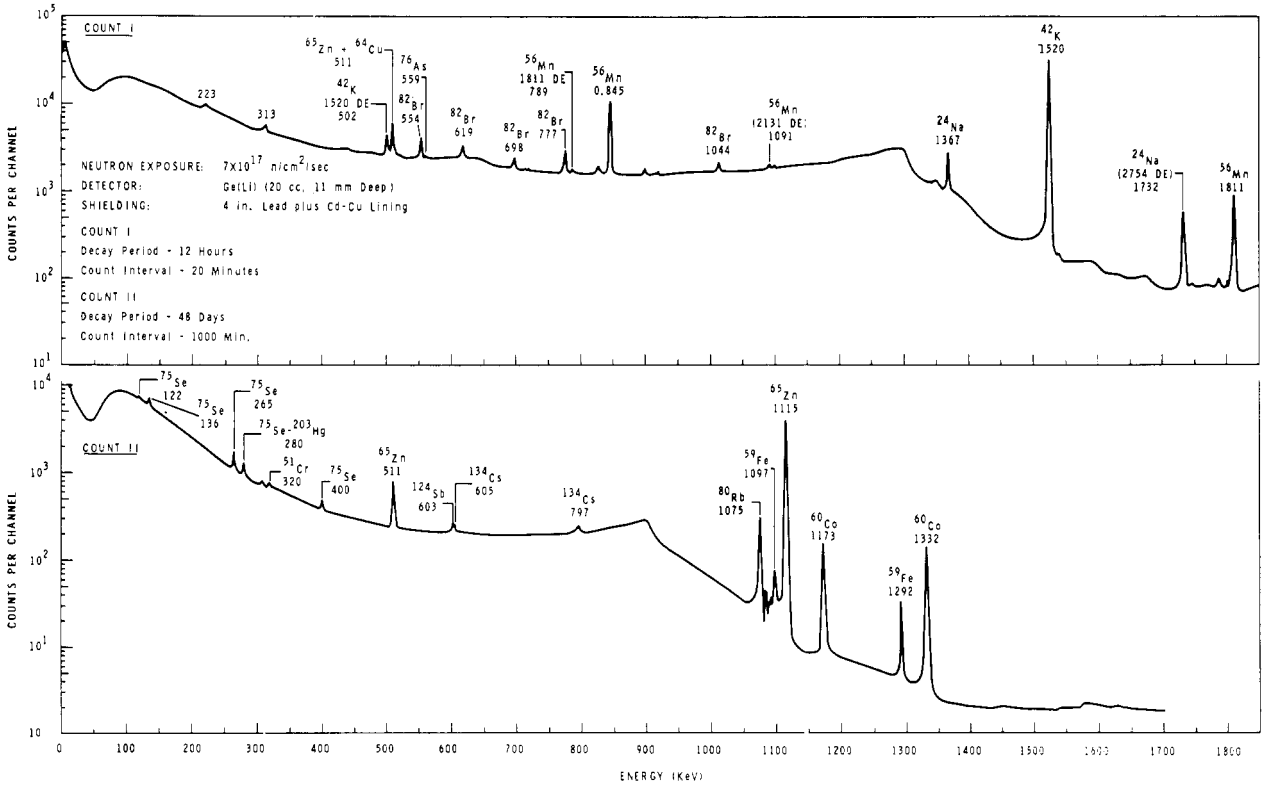

Figure 1. Gamma spectra of neutron-activated lima bean

tion is then determined from the 280-k.e.v. photopeak after ^{75}Se correction. Since ^{32}P is a pure beta emitter, no characteristic γ-ray peaks appear in the spectrum; however, a large bremsstrahlung continuum is produced. To measure this component a portion of the spectrum free of γ-ray peaks is chosen, a Compton correction for higher energy γ-rays is made, and the corrected counts are then compared to those observed from a standard irradiated phosphorus source. Both ^{64}Cu and ^{65}Zn have 511-k.e.v. γ-rays; hence the counts measured in the 511-k.e.v. peak by the first 20-minute counts are due to both ^{64}Cu and ^{65}Zn. When the 1000-minute count is taken after 8 weeks' decay, the ^{64}Cu has completely decayed and the 511 peak is due entirely to ^{65}Zn. The ^{64}Cu 511 peak can then be corrected for the ^{65}Zn contribution. Where the concentration of a trace element was below detection limits, as was the case for many of the ^{76}As measurements, "less than" values were reported. These values were obtained by choosing a counting rate which if present could have been measured above the background plus Compton level with a statistical error of $\pm 50\%$. This value was then substituted into Equation 1. The resulting concentration was reported as a "less than" value.

RESULTS AND DISCUSSION

Table II shows the elemental concentrations observed in the various plant samples studied. Standard deviations were calculated in the usual manner (Friedlander *et al.*, 1964). Three samples of rice and barley, from the same container, were analyzed to indicate the average deviation of the mean for the method.

Comparison of replicate samples showed the average deviation about the mean to be less than 10% for Se, Fe, Zn, Mn, and Cu and less than 25% for the remainder of the elements. In the case of Co, Cs, Cr, P, and Hg the calculated standard deviations based on counting statistics account for the observed variations. The relatively high deviations of Br, Rb, Na, and K cannot be accounted for by statistical considerations. Since these elements are usually in a highly mobile form, slight variation in growing conditions and/or processing could possibly account for these differences. Investigators such as Neufield (1936) and Schrenk (1964) have shown that variations of a factor of 5 in trace element concentrations are possible for the same type of grain, depending on where and how the samples were grown. The elemental concentrations observed in this study were in the same range as published compilations (Mattice, 1950; Neufield, 1936; Wooster and Black, 1955).

Rather large statistical variations occur with some of the Co, Cs, Se, and Hg concentrations. In the case of Co and Cs the high statistical deviations are due to the lack of a sufficient number of counts from the neutron-activated daughter products. The high statistical deviation associated with Cr and Se is related to a low count rate, but of more importance is the large Compton correction associated with higher energy γ-rays and bremsstrahlung from ^{32}P (Table I). Hg's high statistical deviation is caused by the above-mentioned Compton and bremsstrahlung corrections and also the necessity of correcting for the overlap of the ^{75}Se spectrum. ^{65}Zn interference to the ^{64}Cu measurement was in all cases less than 1% of the ^{64}Cu

Table II. Elemental Concentration in Plant Tissue

	Sample Wet Weight, Grams										
	Rice[a,b]				Barley[a,c]		Raisin[d]	Lima Bean[e]	Pea[f]	Apple[g]	Pear[h]
	0.438	0.490	0.442	0.527	0.469	0.408	0.576	1.365	0.463	1.606	3.317
	NANOGRAMS/GRAM										
Co	27 ± 2	20 ± 2	18 ± 2	3 ± 0.5	2 ± 0.5	1.2 ± 0.5	10 ± 0.5	58 ± 2	128 ±	21.9 ± 0.3	10 + 0.2
Cs	4 ± 1	4 ± 1	3 ± 1	<1	<1	<1	54 ± 2	18 ± 1	<1	<1	<1
Cr	35 ± 10	33 ± 10	27 + 10	19 ± 12	16 ± 11	30 ± 13	75 ± 11	27 ± 10	34 + 11	37 ± 1	29 ± 1
Se	113 ± 7	115 ± 7	90 ± 7	50 ± 2	64 ± 3	58 + 3	<1	61 ± 4	<1	22 ± 7	21 ± 7
Hg	62 ± 15	59 ± 15	66 ± 15	117 ± 30	58 ± 26	90 ± 30	63 ± 18	36 ± 16	25 ± 15	20 ± 3	13 ± 3
As	<100	<100	<100	<100	<100	<100	<100	<100	<100	18 ± 6	46 ± 6
	MICROGRAMS/GRAM										
Fe	4.7 ± 0.7	4.2 ± 0.6	4.2 ± 0.6	11.2 ± 1	12.3 ± 1	11.9 ± 1	27.5 ± 1	34.2 ± 1	33.5 ± 1	2.1 ± 0.3	1.6 ± 0.2
Rb	3.1 ± 2	2.9 ± 0.2	2.0 + 0.2	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	15.6 ± 0.2	19.9 ± 0.8	0.5 ± 0.1	0.9 + 0.1	0.5 ± <0.1
Zn	18.3 + 0.2	19.7 ± 0.2	18.1 ± 0.2	14.6 ± 0.7	14.9 ± 0.8	15.1 ± 0.8	1.5 ± 0.2	23 ± 0.5	22 ± 0.6	24 ± 0.4	1.4 ± 0.2
Mn	23.8 ± 0.2	22.9 ± 0.2	23.2 ± 0.2	16.8 ± 0.1	16.2 ± 0.1	17.0 ± 0.1	4.2 ± 0.1	11.5 + 4	23.3 ± 0.2	0.3 ± 0.07	0.8 + 0.04
Na	29.1 ± 0.4	29.6 ± 0.3	26.9 ± 0.3	40 ± 2	58 ± 3	39 + 2	118 ± 1	78 ± 2	78 ± 2	14 ± 1.1	7.6 + 0.4
K	1460 + 20	1681 ± 18	1699 ± 8	1865 ± 10	2211 ± 11	2008 + 12	7200 ± 10	11490 ± 441	7637 ± 70	1187 ± 5	1064 ± 2
Cu	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	2.8 ± 0.2	3.0 ± 0.2	2.4 ± 0.2	1 ± 0.4	4.9 ± 0.2	5.3 ± 0.1	0.3 ± 0.05	0.7 ± 0.1
Br	7.1 ± 1	5.9 ± 0.2	6.0 ± 0.2	4.4 ± 0.3	6.0 ± 0.4	4.8 ± 0.2	8.1 ± 0.2	2.4 ± 0.2	18.3 ± 0.7	<0.05	<0.05
P	345 + 50	215 ± 42	400 ± 56	2400 ± 300	2375 ± 290	2164 ± 285	1699 ± 210	4081 ± 365	4110 ± 210	180 ± 15	130 ± 15

[a] Replicate samples taken from same container.
[b] Rice, Blue Rose, Centennial brand.
[c] Barley, Pearl Barley, Centennial brand.
[d] Raisins, SunMaid.
[e] Lima beans, dried, Centennial brand.
[f] Pea, dried and split.
[g] Apple, fresh less skin and core.
[h] Pear, fresh less skin and core.

counting rate. Since these measurements were made with a 1000-minute count, it would seem unrealistic to attempt to improve the statistics by a longer counting period. However, the efficiency and resolution of the Ge(Li) spectrometers are constantly being improved and detectors with as much as 50% better efficiency and 30% better resolution (measured at 1.33 m.e.v.) than the detector used in this study are now commercially available. Such an improvement in the detector would increase the sensitivity by increasing the counts in the photopeak and reducing the channels over which they are spread.

An important feature of this analytical method is that a large number of what are now considered essential trace elements can be measured simultaneously. Such measurements should help to establish the physiological significance of these and other trace elements such as Cr, Se, Hg, As, and Rb in life processes.

CONCLUSIONS

The actual working time involved in sample preparation and analysis is relatively small, since it involves only freeze-drying, irradiation, and counting of the sample. The mathematical reduction of the count rate data from the spectrum can be handled manually in about 1 hour for 15 elements. Because a large group of trace elements can be measured simultaneously, the direct applicability of this method to all manner of plant samples appears certain and will be extremely useful.

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