Extraction Procedure for Quantitative Determination of Six Elements in Plant Tissue

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A method is described for the extraction and determination of total calcium, magnesium, potassium, manganese, copper, and zinc in plant tissue. The tissue is extracted with 0.1*M* ammonium EDTA, filtered, and the filtrate analyzed using atomic absorption techniques. The results obtained using this procedure agree with those obtained after dry ashing the samples. The procedure described seems ideal for use in a "quick test" tissue analysis laboratory similar to the soil test laboratories operated by many states.

A previous paper (Greweling, 1962) described the determination of total calcium, magnesium, and potassium in dried plant tissue by extracting the tissue with sodium or ammonium salts of (ethylenedinitrilo)tetraacetic acid (sometimes called ethylenediaminetetraacetic acid, EDTA) and measuring the concentration of the metals in the extract with a flame emission spectrophotometer. Spectrographic analysis of the extracts showed that copper, manganese, and zinc are also quantitatively extracted, while aluminum, iron, and phosphorus are only partially extracted using this procedure. Analytical procedures are described in this paper for determination of calcium, magnesium, potassium, manganese, zinc, and copper in EDTA extracts of plant tissue. Results of analysis of several types of plant tissue using EDTA extraction procedure are compared to results obtained after dry ashing the plant tissue.

Reagents and Apparatus

Ammonium EDTA Solution. A 1.0*M* solution was prepared by dissolving 292 grams of reagent grade EDTA in about 600 ml. of demineralized water containing enough NH_4OH to dissolve the acid completely. Additional NH_4OH was added to raise the pH to slightly above 9 and the resulting solution was diluted to 1 liter. This solution was diluted with demineralized water to produce 0.1*M* NH_4EDTA as required. Polypropylene storage containers were used to minimize contamination and to preserve the EDTA. Storage of EDTA solutions in glass reduces the efficiency of this reagent in relieving chemical interferences possibly because of extraction of silica from the glass. Stock solutions of the various elements were prepared as shown in Table I. From these stock solutions working standards were prepared in the appropriate solvents as shown in Table II.

Direct Reading Spectrograph. An Applied Research Laboratories 1.5-meter Quantometer, utilizing a rotating disk solution technique, was used for the analysis of plant tissue ash and ashed EDTA extracts prepared from the plant tissue. The technique used was similar to that described by Kenworthy (1960). Operating parameters are listed in Table III. Using this instrument, the EDTA extraction technique offered no advantage because of the need to evaporate and ash the extracts prior to analysis. This procedure was used only to determine whether the elements of interest were completely extracted by EDTA.

Atomic Absorption Spectrophotometer. A Perkin-Elmer Model 214 instrument was used. The instrument was operated according to manufacturer's instructions (Perkin-Elmer Corp., 1964). More recent results obtained using a Perkin-Elmer Model 303 instrument agree well with those obtained using the Model 214.

Procedure

Extraction of Plant Tissue. The plant tissue was dried at 70° C., ground to pass a 20-mesh sieve, and dried again just before sampling. A 0.25-gram sample was weighed into a 50-ml. container, 20 ml. of 0.1*M* NH_4EDTA solution was added, and the mixture was shaken briefly and then allowed to stand overnight. The next morning the mixture was shaken for 30 minutes on a reciprocating shaking machine before being filtered through Whatman No. 31 filter paper. To minimize contamination, polypropylene containers were used for extraction and storage of the extracts and polypropylene funnels were used for filtration.

Ashing of Plant Tissue. A 0.5-gram sample of dried, ground plant tissue was weighed into a platinum or fused silica crucible and ashed overnight at 500° C. The resulting ash was dissolved in a solvent appropriate for the analytical equipment to be used. For atomic absorption analysis the ash was dissolved in 2 ml. of 1 to 1 HCl, transferred to a 10-ml. volumetric flask, and the solution diluted to the 10-ml. volume with demineralized water. For spectrometric analysis the solvent was 1.5*N* HCl containing 200 μg. of Ni per ml. as an internal standard and 5000 μg. of Li per ml. as a radiation buffer.

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Table I. Preparation of Stock Solutions

Element	Chemical	Weight, G.	Solvent[a]	Final Concentration of Element, μg./Ml.
Calcium	$CaCO_3$	2.497	Minimum HCl	1000
Magnesium	Mg	0.1000	Minimum HCl	100
Potassium	KCl	1.907	H_2O	1000
Zinc	Zn	1.000	Minimum HCl	1000
Manganese	Mn	1.000	Minimum HCl	1000
Copper	Cu	0.1000	Minimum HCl	100

[a] Final volume of all solutions was 1 liter.

Table II. Preparation of Standards for Atomic Absorption Analysis

Element	Concentration Range, μg./Ml.	Solvent	Analytical Wavelength, A.
Calcium[a]	0–20	0.1*M* EDTA	4227
Calcium[b]	0–20	0.1*N* HCl + 10,000 μg. La/ml.	
Magnesium[a]	0–3	0.1*M* EDTA	2852
Magnesium[b]	0–3	0.1*N* HCl + 10,000 μg. La/ml.	
Potassium[a]	0–20	0.1*M* EDTA	7665
Potassium[b]	0–20	0.1*N* HCl + 10,000 μg. La/ml.	
Zinc[a]	0–3	0.1*M* EDTA	2138
Zinc[b]	0–3	0.1*N* HCl	
Manganese[a]	0–5	0.1*M* EDTA	2794
Manganese[b]	0–5	0.1*N* HCl	
Copper[a]	0–3	0.1*M* EDTA	3247
Copper[b]	0–3	0.1*N* HCl	

[a] Standards for extracted samples. [b] Standards for ashed samples.

Table III. Spectrometer Operating Conditions

Power source.	ARL Multisource, Model 5700.
Output voltage.	900 volts.
Capacitance.	2 microfarads.
Inductance.	50 microhenries.
Resistance.	Residual.
Sample electrode.	High purity graphite disk 0.492-inch diameter, 0.200 inch thick (National Carbon Co., No. L-4072, AGKSP).
Counter electrode.	High purity graphite rod, 3/16 inch diameter (National Carbon Co., No. L-3806 AGKSP).
Sample holder.	Porcelain combustion boat.
Analytical gap.	4 mm.
Excitation procedure.	60-second preburn followed by approximately 30 seconds of analytical burn.

Element	Wavelength	Order
Ni (internal standard)	3414.8	1
Ca	3006.9	1
K	4044.1	1
Mg	2781.4	1
Zn	2138.6	1
Mn	2949.2	1
Fe	2599.4	1
Cu	3274.0	1
B	2496.8	2
Al	3092.7	1
Sr	4607.3	1

Analysis of EDTA Extracts. With the exception of copper, the various metals in the EDTA extracts were determined directly by atomic absorption techniques. Any necessary dilutions were made with 0.1*M* EDTA. Calibration curves were established using the EDTA working standards listed in Table II. The concentration of copper in the EDTA extracts from most of the plant tissue samples was too small to be measured directly with the atomic absorption equipment originally available. An increase in sensitivity by a factor of about 5 was obtained by extracting the copper into methyl isobutyl ketone essentially as described by Allan (1961). A 5-ml. aliquot of the EDTA extract was pipetted into an acid-washed borosilicate glass centrifuge tube, then 2 ml. of a 1% solution of ammonium pyrrolidine dithiocarbamate and 5 ml. of methyl isobutyl ketone were added. After being shaken for half an hour to extract copper into the organic phase, tubes were allowed to stand until the phases separated. The concentration of copper in the methyl isobutyl ketone was determined from a calibration curve after absorbance of the sample, and that of a series of copper standards extracted from EDTA solution with methyl isobutyl ketone and ammonium pyrrolidine dithiocarbamate was measured. More recent tests have shown that copper can be determined directly in EDTA extracts if a Boling three-slotted burner head is used with either the Model 214 or Perkin-Elmer Model 303 atomic absorption spectrophotometer equipped with a recorder readout and scale expansion, and this procedure is recommended for routine use.

Analysis of Plant Ash Solutions. The plant ash solutions were diluted as necessary with 0.1*N* HCl before analysis. Calibration curves were established

Table IV. Element Recovered from Plant Tissue

Sample	Spectrometer		Atomic Absorption		A.S.H.S. (Kenworthy *et al.*, 1956) Method of Analysis	
	Ashed	Extracted[a]	Ashed	Extracted	Chemical	Spectrographic
			Calcium,[b] C.V. 2.8%[c]			
Turnip greens	2.32	2.46	2.41	2.47		
Red pine needles	0.22	0.16	0.29	0.21		
Sorghum[d]	0.34	0.46	0.42	0.37		
Corn grain		0.03	0.02	0.05		
Ladino clover[d] 4276	1.42	1.49	1.52	1.47		
Ladino clover[d] 5509	1.45	1.43	1.57	1.51		
Apple leaves	1.09	1.03	1.21	1.21	1.12	1.31
Cherry leaves	2.50	2.57	2.94	2.71	2.90	2.99
Citrus leaves	3.50	3.40	3.67	3.69	3.67	3.58
Peach leaves	1.90	2.00	1.97	1.95	1.94	2.04
			Magnesium,[b] C.V. 4.1%			
Turnip greens	0.31	0.32	0.30	0.33		
Red pine needles	0.06	0.06	0.07	0.09		
Sorghum[d]	0.23	0.22	0.24	0.24		
Corn grain	0.10	0.10	0.11	0.11		
Ladino clover[d] 4276	0.26	0.26	0.28	0.27		
Ladino clover[d] 5509	0.32	0.32	0.33	0.32		
Apple leaves	0.33	0.34	0.34	0.33	0.36	0.36
Cherry leaves	0.87	0.91	0.93	0.88	0.85	1.01
Citrus leaves	0.30	0.31	0.31	0.32	0.35	0.30
Peach leaves	0.50	0.52	0.51	0.51	0.51	0.52
			Potassium,[b,e] C.V. 5.7%			
Turnip greens			4.24	4.18		
Red pine needles			0.32	0.34		
Sorghum[d]			1.31	1.32		
Corn grain			0.32	0.33		
Ladino clover[d] 4276			2.21	2.21		
Ladino clover[d] 5509			1.54	1.50		
Apple leaves			1.26	1.11	1.16	1.18
Cherry leaves			1.76	1.55	1.71	1.58
Citrus leaves			1.06	1.04	1.04	1.12
Peach leaves			2.16	2.02	2.25	2.07

[a] 60-minute extraction.
[b] Results given in per cent.
[c] Coefficients of variation determined by extracting six replicates of three samples (turnip greens, sorghum, and Ladino clover 5509). Duplicate absorption readings were recorded for each replicate. Boling burner was used for Cu, Mn, and Zn; standard burner for Ca, Mg, and K.

for each element in 0.1*N* HCl. To protect Ca from interference by ions such as phosphate, sufficient $LaCl_3$ solution was added during dilution of plant ash solutions for Ca determination to maintain a La concentration of 10,000 μg. per ml. in the solutions being analyzed. Because the sample solution diluted for Ca determination was also used for Mg and K determination, $LaCl_3$ solution was added to the standard Mg and K solutions, as well as to the standard Ca solutions, to provide the same La concentration in the standards as in the sample solutions.

Results and Discussion

The analytical results comparing values for the concentration of Ca, Mg, K, Cu, Mn, and Zn obtained using ashing and extraction techniques for analysis of several different kinds of plant tissue are listed in Table IV. The values are averages for three ashings or extractions of each sample. The range of results obtained by the American Society for Horticultural Science referee analysis (Kenworthy *et al.*, 1956) of four of the samples is also listed. It is not within the scope of this paper to compare the results obtained by several analytical instruments, but rather to demonstrate that, with a given instrument, the results obtained using the extraction procedure agree with those obtained using a dry-ashing procedure, thus indicating quantitative extraction of the different elements. However, in fact, the results obtained using different instruments do agree within acceptable limits.

As shown in Table IV, the results of Ca determination in the plant tissue samples using EDTA extraction procedure agree closely with those obtained using the dry-ashing procedure in which a high concentration of La was used to prevent interference caused by formation

Table IV. Continued

Sample	Spectrometer		Atomic Absorption		A.S.H. S. (Kenworthy *et al.*, 1956) Method of Analysis	
	Ashed	Extracted[a]	Ashed	Extracted	Chemical	Spectrographic
			Copper,[f] C.V. 3.5%			
Turnip greens	12	14	14	13		
Red pine needles	6	7	7	8		
Sorghum[d]	13	...	11	10		
Corn grain	6	6	6	9		
Ladino clover[d] 4276	15	14	14	14		
Ladino clover[d] 5509	18	15	15	15		
Apple leaves	13	14	13	13	13	15
Cherry leaves	306	298	298	287	307	188
Citrus leaves	35	32	32	33	32	35
Peach leaves	21	22	22	23	20	22
			Manganese,[f] C.V. 1.6%			
Turnip greens	103	108	114	114		
Red pine needles	...	...	715	726		
Sorghum[d]	40	36	46	51		
Corn grain	10	11	7	9		
Ladino clover[d] 4276	53	53	57	55		
Ladino clover[d] 5509	63	58	64	67		
Apple leaves	98	93	106	108	107	102
Cherry leaves	88	88	88	70	109	97
Citrus leaves	36	36	28	31	32	35
Peach leaves	78	78	87	81	77	83
			Zinc,[f] C.V. 4.7%			
Turnip greens	35	37	35	33		
Red pine needles	38	38	41	41		
Sorghum[d]	36	31	32	35		
Corn grain	21	21	23	23		
Ladino clover[d] 4276	35	36	34	36		
Ladino clover[d] 5509	36	36	32	33		
Apple leaves	23	23	22	23	23	34
Cherry leaves	28	31	35	28	44	38
Citrus leaves	86	72	75	74	73	90
Peach leaves	31	28	36	37	33	31

[d] Entire above ground plant.
[e] Results of K analysis using the spectrometer are omitted because the precision was poor.
[f] Results given in p.p.m.

of refractory compounds in the flame with elements such as phosphorus, aluminum, and silicon. Greweling (1962) has shown that, in flame emission analysis, Ca complexed with EDTA is protected from interference of this type. The EDTA apparently has the same effect in atomic absorption analysis. In addition, the elements most likely to cause this type of interference in the determination of Ca are not completely extracted from the tissue by EDTA.

As Greweling (1962) has shown, the time required for complete extraction of Ca with ammonium EDTA varies with different types of plant tissue. Although one-half hour is sufficient time for complete extraction of Ca from most plant samples, low values for Ca were obtained when the red pine needles and sorghum samples were extracted for only half an hour. The samples for spectrometric analysis were extracted for 1 hour, but overnight extraction is recommended as a more certain procedure for the extraction of Ca especially when evergreen tissue is involved. Use of sodium EDTA reduced the time required for extraction of Ca (Greweling, 1962), but caused high instrument noise in the determination of K.

The concentrations of Mn, Zn, and Cu in the EDTA extracts were very low and using the standard burner head, the EDTA extracts had to be diluted 1 to 1 in order to reduce noise in the determination of Mn and Zn. After the 1 to 1 dilution, the concentration of Mn in an EDTA extract from the sorghum sample, for example, as measured using the atomic absorption spectrophotometer was only 0.33 p.p.m., corresponding to an absorbance of 0.0137. The signal to noise ratio in Mn analysis by atomic absorption is very high, so that absorption readings can be made $\pm 0.1\%$ absorption. Such a variation in the per cent absorption recorded for the EDTA extract from the sorghum sample

would correspond to a range of from 50 to 54 p.p.m. in the apparent Mn content of the tissue. Apparently, use of a ratio of tissue to EDTA greater than the 1 gram to 80 ml. described in this study should provide greater concentration of these elements in the extracts and more precise measure of the concentrations of the elements in the tissue. Analysis of EDTA extracts obtained using a greater ratio of tissue to EDTA, however, gave low results unless the extracts were diluted with EDTA. The source of the apparent interference when more concentrated extracts were analyzed is not known. However, later studies showed that the extracts can be analyzed without dilution and with greater sensitivity using the three-slotted Boling burner head. These results suggest that the problem encountered using the standard burner head and undiluted extracts may be related to burner clogging caused by organic matter extracted from the plants. Using the Boling burner head, direct measurement of Cu, Mn, and Zn is possible in undiluted extracts of most plant tissue samples. A more sensitive measurement of Zn and Mn concentrations might also be obtained by extraction with an organic solvent. However, several attempts to extract Zn from EDTA extracts using methyl isobutyl ketone and ammonium pyrrolidine dithiocarbamate failed even after acidifying the EDTA extract.

Owing to the very low concentrations of Mn, Zn, and Cu in the EDTA extracts, the coefficient of variation of the concentration of these elements in the different plant tissue samples as measured by standard burner atomic absorption analysis of the EDTA extracts was relatively high. The average coefficient of variation for analysis of the different tissue samples was 6% for Cu, 8% for Zn, and 4% for Mn. Nevertheless, the results obtained using the EDTA extraction technique agreed closely with those obtained after dry ashing the samples. Although greater precision can be obtained by dry ashing, using larger samples, and dissolving the ash in smaller volumes, the results obtained using the EDTA extraction method are sufficiently precise for use in a "quick test" tissue analysis laboratory and the precision is improved if a Boling burner head is used. The Boling burner was used to collect data for the coefficients of variation for Cu, Mn, and Zn listed in Table IV.

Elements Not Completely Extracted

Iron. The amount of iron extracted ranged from 3 to 40% of the total with a mean of about 10% for the types of tissue tested. Possibly, only ferrous iron was being extracted; however, the addition of a reducing agent ($NH_2OH \cdot HCl$) to the extraction solution failed to increase significantly the amount of iron extracted. Tests showed that NH_2OH does reduce ferric iron when both are added to the EDTA extraction solution. This seems to indicate that the unextracted iron is held very tightly by the plant tissue, a conclusion which is supported by the work of Knezek and Maier (1966).

Aluminum. The per cent of aluminum extracted ranged from 7 to 29 with a mean of about 20%. Since aluminum is not an essential plant nutrient and interferes in the flame emission and atomic absorption determination of the alkaline earth elements, this incomplete extraction may be considered beneficial.

Phosphorus. The amount of phosphorus extracted ranged from 44 to 83% of the total, the mean being slightly over 60% in the plants examined. No attempt has been made to correlate the amount of phosphorus extracted with plant growth. Tests on a few samples showed a fair correlation between EDTA-extractable phosphorus and the amounts of phosphorus extracted by the dilute acetic acid procedure described by Johnson and Ullrich (1959).

Boron. EDTA-extractable boron varied between 21 and 92% of the total for the tissue tested. The mean per cent extracted was 60. The factors governing the amount of boron extracted have not been investigated and no attempt has been made to correlate EDTA-extractable boron with plant growth.

Strontium. Preliminary spectrometric analysis indicates that strontium is probably completely extracted by this technique. However, because a strontium hollow cathode lamp was not on hand, no atomic absorption data were obtained. Spectrometric determination of strontium suffered from a lack of precision because the lithium used as a radiation buffer contained relatively large amounts of strontium.

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