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Determination of Mineral Elements in Plant Tissues Using Trichloroacetic Acid Extraction

Glen E. Leggett* and Dale T. Westermann

An extraction method is described for quantitatively determining Mg, K, Na, Zn, and Mn in plant tissues. The tissue is extracted with 2% trichloroacetic acid and the elements are determined on the filtrate by atomic absorption spectrophotometry. Results compare favorably with those determined after wet ashing with nitric and perchloric acids. In addition, trichloroacetic acid extracts the same fraction of P as does acetic acid and quantitatively extracts total Ca from plant tissues not high in oxalate.

The mineral content of plant samples is usually determined by analyzing the liquid phase after dry ashing or wet ashing ground dried plant materials. The procedures and the problems of fume disposal and special laboratory equipment required for the methods have been discussed (Johnson and Ulrich, 1959). Nicholas (1951) surveyed the literature on extraction methods for tissue analysis in determining the nutrient status of various crops. Many reports indicate good correlations between nutrients extracted and fertilizer applications, but only a few compare the extracted values to the total content. Nicholas (1948a,b) extracted plant tissue with acetate, citrate, malonate, and succinate buffers (pH 4.8). The concentrations determined by extraction were correlated with the total concentrations in the tissue, but the two sets of values were not directly compared. Greweling (1962) and Baker and Greweling (1967) analyzed the EDTA extracts from a variety of plant tissues for several mineral elements and compared the results with those obtained after dry ashing. In most cases the extracted and ashed values agreed closely.

Analysis of acetic acid extracts of plant tissues for Zn and Mn indicated that extraction of these elements was essentially complete from most but not all plant materials. Consequently, a stronger acid, trichloroacetic, was used and the number of elements determined extended to include Ca, Mg, K, Na, Cu, Fe, and P. This report deals with the use of trichloroacetic acid for extracting the total contents of several mineral elements from various plant tissues and a study of some of the factors affecting its use.

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METHODS AND MATERIALS

Plant Samples. Forty-two samples representing 20 different crops (Table I) were analyzed for their total content of several mineral elements by wet ashing and for the amount of the various constituents extracted in various solutions. All samples were washed in demineralized water, dried at about 60°, and ground in an all-steel Wiley mill to pass a 40-mesh stainless steel sieve. The ground material was redried for at least 2 hr at 60° before weighing out samples for analysis.

Extracting Solutions. *Trichloroacetic Acid (TCA) Solution.* A 2% (w/v) solution was prepared by dissolving 20 g of reagent grade TCA in distilled demineralized water and diluting to 1 l.

Acetic Acid (HOAc) Solution. A 2% (v/v) solution was prepared by diluting 20 ml of reagent grade HOAc to 1 l. with distilled demineralized water.

Ammonium EDTA Solution. A 1.0 *M* stock solution was prepared according to the procedure of Baker and Greweling (1967). This solution was diluted with distilled demineralized water to make 0.1 *M* ammonium EDTA as needed.

Extraction of Plant Tissue. Samples (0.5 g) were weighed into wide-mouthed 125-ml linear polyethylene

Table I. Plant Samples Used for Analysis

Crop	Plant part and no. of samples
Alfalfa *(Medicago sativa)*	Stems and leaves (3)
Asparagus *(Asparagus officinalis)*	Fern (1)
Beans *(Phaseolus vulgaris)*	Tops (2)
Garden beet *(Beta vulgaris)*	Leaf blades (2)
Sugarbeet *(Beta vulgaris* L.)	Leaf blades (3)
Brussels sprout *(Brassica oleracea gemmifera)*	Heads (1)
Cabbage *(Brassica oleracea capitata)*	Heads (1)
Corn *(Zea mays)*	Leaves (4), tops (3)
Hops *(Humulus lupulus)*	Leaves (3), cones (1)
Lettuce *(Lactuca sativa* L.)	Leaves (1)
Peppermint *(Mentha piperita* L.)	Shoots (3), stems (1)
Spearmint *(Mentha spicata)*	Stems (1)
Parsley *(Petroselinum crispum latifolium)*	Tops (1)
Pea *(Pisum sativum)*	Vines (1)
Potato *(Solanum tuberosum)*	Tops (3)
Rhubarb *(Rheum rhaponticum)*	Leaf blades (1)
Sorghum *(Sorghum vulgare)*	Tops (3)
Spinach *(Spinacia oleracea)*	Leaves (1)
Swiss chard *(Beta vulgaris cicla)*	Leaf stalks (1)
Wheat *(Triticum aestivum)*	Ripe straw (1)

bottles fitted with polyethylene-lined caps. Each sample received one spoonful (approximately 1 g) of activated carbon and 50 ml of extracting solution. Samples were shaken on a reciprocating shaker for 16 hr at 25°, or for prescribed periods of time. Some samples were heated at 60° in a bath of running tap water; these samples were shaken intermittently by hand. The bottles containing the heated samples were fitted with polypropylene caps to withstand the pressure and to prevent evaporation. All samples were filtered through Whatman No. 50 paper. To avoid having carbon appear in the filtrate the samples were shaken vigorously immediately before pouring them rapidly onto the filter.

Wet Ashing of Plant Tissue. Samples (0.5 g) of dried ground plant material were weighed into 100-ml Kjeldahl flasks containing a glass bead. The samples were predigested with 5 ml of HNO_3, cooled, and digested to fumes of $HClO_4$ after adding 5 ml of a mixture of HNO_3 and $HClO_4$ (3 + 1). After digestion, about 40 ml of distilled demineralized water was added to each flask and the contents were brought to near boiling to ensure complete dissolution of sample except for silica. Samples were cooled, diluted to 50 ml, and filtered through Whatman No. 50 paper.

Chemical Determinations. The metal cations in the extracts and digests were determined by atomic absorption spectrophotometry. Cu, Zn, Mn, and Fe were determined directly on the filtrates, whereas Ca, Mg, K, and Na were determined after diluting the filtrate (1 + 24) with a solution containing 1% La and 5% HCl. P was determined on a suitable aliquot of the undiluted filtrate by the vanadomolybdate method (Kitson and Mellon, 1944).

RESULTS AND DISCUSSION

Mineral concentrations determined after wet ashing and TCA extraction (16 hr, 25°) are compared in Figure 1. Extraction of Mg, K, Na, Zn, and Mn is quantitative for all 42 samples. The extraction of Ca was complete for most crops; but from beet leaves, rhubarb leaves, spinach, and Swiss chard it averaged 65% and tended to be low from potato tops (87%) and hop leaves (94%). Almost no Ca was extracted from beets, rhubarb, spinach, and chard by HOAc, and increasing the TCA concentration to 3% increased the Ca extracted from these materials to only 80% of the total. These crops contain large amounts of oxalate, thus it was assumed that Ca was immobilized as the oxalate either before or during the extraction. Except for these eight samples, the agreement between Ca extracted with TCA and total Ca is good.

The values reported for Cu should be considered tentative. The Cu concentrations in most samples analyzed approached the limits of determination for the techniques used here. Moreover, a variable blank for Cu was encoun-

Table II. The Effect of Time and Temperature on the Extraction of Mineral Elements from Plant Tissue by TCA Solution

	Mineral concentrations determined by various procedures[a]											
	Ca, %			Mg, %			K, %			P, %		
	Total	TCA	TCA − H	Total	TCA	TCA − H	Total	TCA	TCA − H	Total	TCA	TCA − H
Hop leaves	4.18	4.02	4.10	1.58	1.56	1.51	1.16	1.24	1.19	0.276	0.118	0.111
Corn leaves	0.90	0.91	0.94	0.64	0.63	0.67	2.46	2.45	2.47	0.323	0.169	0.192
Alfalfa	1.51	1.35	1.43	0.43	0.42	0.45	3.61	3.52	3.62	0.390	0.162	0.169
Bean tops	2.62	2.55	2.66	0.49	0.48	0.50	3.62	3.53	3.66	0.324	0.113	0.118
Sorghum tops	0.46	0.46	0.47	0.21	0.21	0.20	1.62	1.63	1.66	0.376	0.173	0.175
Potato tops	1.98	1.70	1.84	0.71	0.70	0.71	5.40	5.37	5.52	0.574	0.319	0.315
Sugarbeet leaves	1.47	0.56	0.92	0.91	0.89	0.91	2.65	2.52	2.59	0.435	0.313	0.312
	Zn, ppm			Mn, ppm			Cu, ppm			Fe, ppm		
	Total	TCA	TCA − H	Total	TCA	TCA − H	Total	TCA	TCA − H	Total	TCA	TCA − H
Hop leaves	22	20	21	84	82	81	9	6	7	410	51	82
Corn leaves	13	12	13	236	234	234	31	22	26	203		104
Alfalfa	23	25	24	40	40	38	12	9	9	80		26
Bean tops	25	25	24	84	81	81	8	5	6	458		45
Sorghum tops	34	32	31	44	40	39	5	3	4	470	70	75
Potato tops	56	55	57	58	57	54	10	6	7	282	42	64
Sugarbeet leaves	62	62	60	102	101	97	15	12	13	422	115	137

[a]Total determined by wet-ashing, TCA by extracting for 16 hr at 25°, and TCA − H by extracting for 1 hr at 60°.

Table III. Mineral Concentrations in Plant Tissues Determined by Extraction and Wet Ashing

	Mineral concentrations determined by various methods														
	Ca, %					Mg, %					K, %				
	Total	TCA + C	TCA − C	EDTA	HOAc	Total	TCA + C	TCA − C	EDTA	HOAc	Total	TCA + C	TCA − C	EDTA	HOAc
Peppermint	1.38	1.38	1.36	1.40	1.09	0.73	0.72	0.71	0.71	0.63	2.72	2.72	2.73	2.68	2.53
Corn tops	0.31	0.29	0.29	0.30	0.25	0.32	0.29	0.30	0.30	0.29	1.65	1.54	1.65	1.61	1.57
Corn leaves	0.71	0.71	0.71	0.71	0.59	0.82	0.82	0.82	0.81	0.70	3.07	3.08	3.11	3.03	2.96
Corn leaves	1.18	1.18	1.18	1.19	1.03	1.08	1.05	1.05	1.04	1.02	0.99	1.03	1.03	1.01	1.04
Hop leaves	4.18	4.02	3.98	4.18	3.57	1.54	1.50	1.50	1.40	1.51	1.12	1.21	1.22	1.10	1.18
Sugarbeet leaves	1.47	0.56	0.50	1.46	0.01	0.92	0.90	0.88	0.88	0.87	2.64	2.64	2.67	2.65	2.56
Potato tops	1.64	1.45	1.36	1.63	0.51	0.83	0.82	0.82	0.81	0.75	5.88	5.93	5.95	6.05	5.55
Potato tops	1.98	1.70	1.67	1.97	0.92	0.71	0.70	0.70	0.70	0.65	5.36	5.37	5.33	5.44	4.92
Sorghum tops	0.44	0.44	0.42	0.43	0.37	0.21	0.20	0.19	0.19	0.19	1.53	1.63	1.60	1.55	1.59
Sorghum tops	0.46	0.46	0.45	0.46	0.40	0.21	0.19	0.19	0.19	0.20	1.63	1.64	1.63	1.65	1.63
	Zn, ppm					Mn, ppm					Cu, ppm				
	Total	TCA + C	TCA − C	EDTA	HOAc	Total	TCA + C	TCA − C	EDTA	HOAc	Total	TCA + C	TCA − C	EDTA	HOAc
Peppermint	21	21	19	18	15	67	67	65	55	59	10	7		7	2
Corn tops	16	17	16	13	16	50	45	45	38	46	6	3		3	4
Corn leaves	7	7	6	5	10	186	187	176	152	190	31	27		24	12
Corn leaves	65	65	64	55	62	141	140	141	119	147	12	8		7	3
Hop leaves	20	21	19	17	18	88	87	85	58	85	9	6		6	4
Sugarbeet leaves	60	63	56	51	52	99	100	96	80	96	14	13		11	6
Potato tops	419	414	415	350	273	96	100	98	84	79	13	8		9	5
Potato tops	54	55	53	48	40	55	55	56	47	48	10	6		6	3
Sorghum tops	46	41	41	34	41	46	41	40	33	42	9	4		4	2
Sorghum tops	32	32	31	27	32	44	39	38	32	40	5	3		3	3

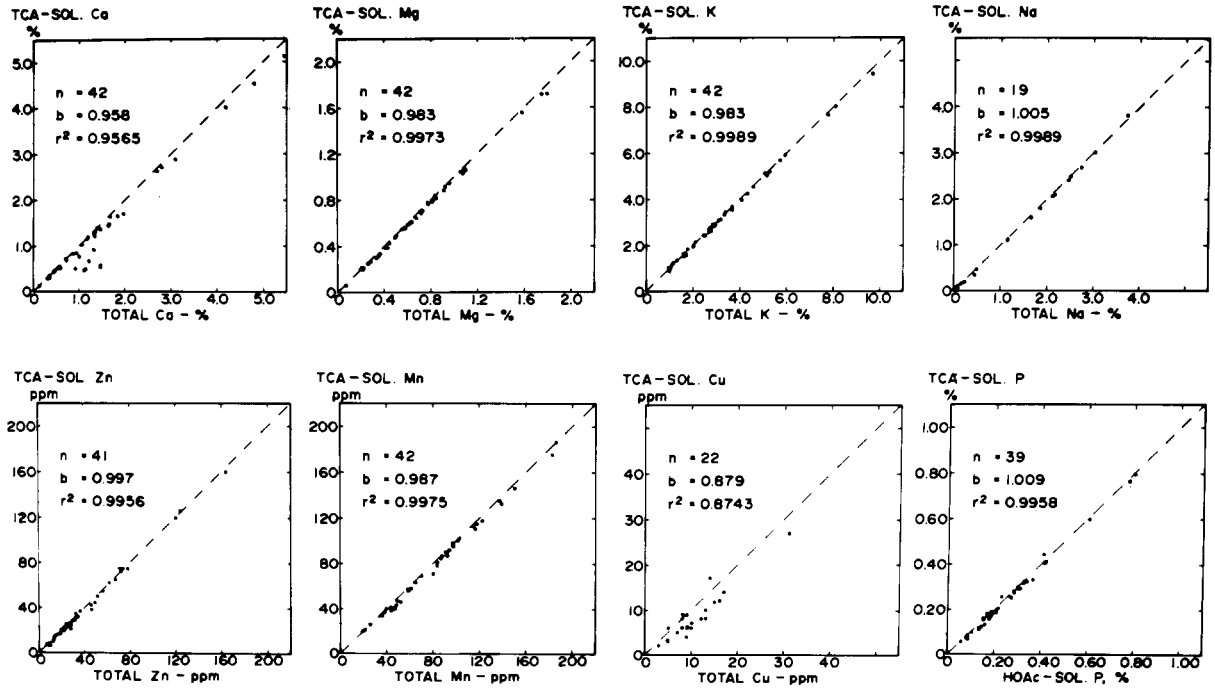

Figure 1. The relationship between TCA-soluble and total Ca, Mg, K, Na, Zn, Mn, Cu and TCA-soluble and HOAc-soluble P in plant tissues. The dashed lines indicate 1:1 slopes.

tered for one set of wet-ashed samples; consequently, the values were discarded.

The Fe extracted by TCA was always less than 40% and usually less than 15% of the total Fe present. There was no obvious relationship between the total and extracted values so they are not presented. These results are similar to those obtained on various crops using EDTA extraction solutions (Baker and Greweling, 1967) and to those determined on tomatoes using other chelating agents (Knezek and Maier, 1966). Limited work indicated that recovery of Fe with TCA was no better from fresh than from dried alfalfa samples.

The results from only the 19 samples that contained more than 0.02% Na are presented in Figure 1. Values smaller than 0.02% were uncertain because of a relatively high blank. Plants containing appreciable Na are beets, spinach, chard, mint, cabbage, and Brussels sprouts.

The extraction of P by TCA was incomplete from all samples tested, but the values obtained were almost identical to HOAc-soluble P (Figure 1). Similar results were reported by Barr and Ulrich (1963). This feature enhances the value of the TCA extraction because of the extensive work relating HOAc-soluble P to the P status of many different crops.

Table IV. The Range and Mean Concentrations for Several Elements Extracted by TCA from Seven Samples and the Coefficients of Variation Calculated from Analysis of Four Replications

Element determined	Range in values: Low	High	Mean	Coefficient of variation, %
	%			
Ca	0.59	4.26	1.70	1.4
Mg	0.41	1.47	0.67	1.4
K	1.20	5.24	2.84	2.9
P	0.101	0.293	0.183	1.4
	ppm			
Zn	10	56	30	2.5
Mn	40	256	94	1.5
Cu	4	24	10	7.3

Samples consisted of one each of sugarbeet leaves, potato tops, sorghum tops, hop leaves, alfalfa, bean tops, and corn leaves.

The results of a separate study indicate that the extraction time can be shortened to 1 hr if the temperature is raised to 60°. For the seven samples listed in Table II, concentrations of Mg, K, P, Zn, Mn, and Cu determined under these conditions were the same as those determined after 16 hr and 25°. Except for sugarbeet leaves, extraction of Ca at 60° was nearly complete from all seven crops tested; the Ca extracted from sugarbeet leaves was higher than that obtained at 16 hr and 25° but was still incomplete.

In view of these data, a 1-hr extraction period at 60° is just as good as the 16-hr extraction at 25° for the seven plant materials used. Unfortunately, data are not available for all samples, but it is likely that the shortened extraction time at 60° would be satisfactory for the other crops used in the study, and investigation on other crops would be warranted.

A separate study was made using ten samples for comparing TCA, HOAc, and EDTA as extracting solutions (16 hr, 25°) for six mineral elements (Table III). The results indicate that TCA was superior to or equal to EDTA in extracting Mg, K, Zn, Mn, and Cu and that, except for Cu, the values compare favorably with those determined after wet ashing. EDTA proved superior to TCA for extracting Ca from sugarbeet leaves, potato tops, and hop leaves. However, Ca determined by the two methods agreed closely for the other crops listed. The low EDTA values given for Zn and Mn may have resulted from a higher viscosity of the EDTA extracts, since these elements were determined by aspirating undiluted extracts directly into the burner.

Values resulting from HOAc extraction were generally low, especially for Ca, Zn, and Cu.

The extractable Fe (not given) was low compared to the totals for all extracting solutions; those for HOAc were near zero.

Phosphorus was not determined on the EDTA extracts because most of them were colored. The amounts extracted with TCA and HOAc were nearly the same for all samples tested, as was shown earlier in Figure 1.

The charcoal (Fisher, C-179) used in this work is no longer available. It was used without purification and gave blanks that were near zero for all elements determined. Rather than use impure charcoal, it is recommended that it be omitted, despite the resulting colored extracts. Analysis of ten samples with and without charcoal (Table III) indicates that charcoal did not affect the recovery of Ca, Mg, K, Zn, and Mn. It is likely that results for Na and Cu would likewise be unaffected. However, for colorimetric determination of P, aliquots must be decolorized before developing the color (Johnson and Ulrich, 1959).

Other work (data not given) indicates significant variation in extractability of some elements from some plant materials. For example, at 25° Mn extraction was incomplete from hop leaves and bean plants after 4 hr, but was complete from the other samples listed in Table II after only 1 hr, and was complete from all samples listed after 1 hr at 60°. Moreover, for sorghum tops and corn leaves, all elements studied, except Fe, were quantitatively extracted in 1 hr at 25°.

An interesting feature of TCA extraction is that extractability is not dependent on element concentration. For example, all of the Mg was extracted in 1 hr at 25° from hop leaves containing 1.75% Mg, as well as from sorghum tops containing only 0.21% Mg. Similarly, the extraction of Zn was quantitative from wheat straw (7 ppm), potato tops (56 ppm), and potato tops (419 ppm). Again, Mn was completely extracted from corn leaves (235 ppm) in 1 hr at 25°, but was only 83% complete from beans (81 ppm) after 1 hr and 95% after 4 hr. Thus, the extractability of the elements seems to be more related to the plant material than to the element concentration.

The precision of the TCA extraction method is well within acceptable limits for the various elements (Table IV). The high coefficient of variation for Cu results from the low concentrations; the limits of determination for this element were approached with the techniques used. Certainly the precision of measurement on the TCA extracts is as good as that for wet ashing. No difficulty in determining Ca in the presence of TCA was encountered, as was reported by Baker *et al.* (1969).

The results of the studies indicate that TCA extraction provides adequate analytical answers for most purposes. The method should prove most useful in laboratories routinely analyzing large number of plant samples for several elements. The procedure is simple and rapid, and it avoids the hazards associated with $HClO_4$ and the special manifolds and fume hoods required in wet ashing. Likewise, it eliminates the transfer of samples and thus decreases the risk of contamination, and also eliminates the need for platinum labware used for removing silica after dry ashing.

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Chromium in Foods in Relation to Biological Activity

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Chromium was determined in foods and in extracts of selected samples of foods using modification of the colorimetric procedure to stabilize color development. Extracts were prepared both by hydrolysis of samples to obtain total chromium and by alcohol extraction. Biological activities of these extracts were determined using the glucose oxidation procedure in the presence of insulin. No significant relationship was found between total chromium and biological activity. However, there was a significant relationship for chromium in alcohol extracts of meats, fungi, seeds, and seafoods, excluding fruits and vegetables. A proposed evaluation of the foods was based on these data.

The occurrence and function of chromium in biological systems have been summarized by Mertz (1969). Among the properties reported was the *in vitro* potentiation of insulin-stimulated oxidation of glucose in the presence of rat fat pad tissue (Roginski *et al.*, 1970, 1971). In phosphate buffer, chromium as an inorganic salt gave some increase in glucose oxidation in this system. However, the rate of oxidation was greatly increased when extracts of Brewers yeast containing chromium were added. On the basis of extracts containing chromium complexed in organic structures capable of restoring serum glucose levels in deficient animals, such compounds became known as the chromium glucose tolerance factor (GTF). As part of the research on chromium distribution, the total chromium in a food sample could be determined by chemical analysis, but only part of that chromium was available for

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