

Flame Photometric Determination of Manganese, Iron, and Copper in Plant Material

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The Beckman Model DU flame spectrophotometer with photomultiplier attachment was utilized to determine trace amounts of manganese, iron, and copper in plant material. The wave lengths used for analyses were: iron, 372 $m\mu$; manganese, 403.3 $m\mu$; and copper, 324.8 $m\mu$. Interfering ions were removed or separated through the use of an 8-hydroxyquinoline differential pH precipitation. A dry ashing technique was used in sample preparation. It was necessary to ash the precipitate from the 8-hydroxyquinoline treatment to reduce flame background. Comparison of the flame procedure with Association of Official Agricultural Chemists procedures showed good results.

CONSIDERABLE DATA appear in the literature concerning flame methods for the analysis of manganese, iron, and copper (4-8, 14). Most of these papers, however, are concerned with siliceous materials, alloys, and other samples of similar nature. Relatively little appears concerning such determinations in plant materials. As the bulk of the material present in siliceous substances and alloys is much different than the mineral composition of plants, sample preparation, flame interferences, and control of sample variables will be different. Spectral lines used for analytical purposes after sample preparation and instrumental conditions may be similar.

The trace element constituents of plant materials were separated before flame analysis in order to eliminate the undesirable effects of the major constituents in the flame. Several methods are available for such separations, however, 8-hydroxyquinoline has been used successfully by a number of workers (1, 9, 10, 12, 13) who then utilized colorimetry for the final step in their procedures. This technique of separation has not been applied to sample preparation for flame photometry previously.

Apparatus

A Beckman Model DU quartz spectrophotometer equipped with a Model 9200 flame photometer attachment and photomultiplier circuit and utilizing an oxygen-acetylene flame was used throughout this investigation.

Reagents

Hydrochloric acid, c.p., concentrated.
Hydrochloric acid, approximately 0.1 *N*.

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Ammonium acetate, 2*N*.
Acetic acid, 2*N*.
8-Hydroxyquinoline, 5%, in 2*N* acetic acid.
Ammonium hydroxide (dilute concentrated 1 to 1).
Aluminum chloride solution (4000 p.p.m. aluminum).

Procedure

Weigh out 2.0-gram samples of plant material and transfer to 50-ml. platinum dishes. Destroy the major portion of the organic material by heating over a low Bunsen flame, being careful not to allow the sample to burst into flame. Place the samples in a muffle furnace at 500° C. for 16 hours. Remove the dishes from the furnace and desiccate until cool. Add 8 ml. of concentrated hydrochloric acid to each ashed sample and heat gently over a low Bunsen flame to effect solution. Transfer the solutions to 400-ml. beakers and rinse the dishes several times with distilled water, using a total volume of 70 ml.

Iron and copper are separated from manganese as follows: Add 0.5 ml. of a solution of aluminum chloride containing 4000 p.p.m. aluminum to each sample in the beakers followed by 10 ml. of a 5% solution of 8-hydroxyquinoline. Ammonium hydroxide (diluted 1 to 1) is then added until the solution has a pH of 1.2 to 1.3. Add 40 ml. of 2*N* ammonium acetate with vigorous stirring, followed by 1 to 1 ammonium hydroxide to give a final pH of 5.1, as determined by a pH meter. Cover the beakers with watch glasses and allow to stand overnight. Remove the precipitate by filter-

ing, under mild suction, on Whatman No. 52 filter paper. Wash the residue from the beakers by rinsing with the filtrate.

Place the precipitate and filter paper in small fused silica crucibles and char the paper over a low Bunsen flame, being careful not to allow the paper to burst into flame. Ignite the precipitate in a muffle furnace and leave at 850° C. for 16 hours. Remove the crucibles from the furnace and desiccate until cool. Transfer the residue in the crucibles to 30-ml. beakers with 3 ml. of concentrated hydrochloric acid. Rinse the crucibles with several small portions of distilled water and add to the 30-ml. beakers so that the final volume is approximately 10 to 12 ml. Place the beakers on a hot plate on medium heat and evaporate the contents just to dryness. Care must be exercised to prevent spattering. Then put the residue in the beaker into solution with 2 ml. of 0.1*N* hydrochloric acid and rinse the beakers thoroughly with sufficient 0.1*N* hydrochloric acid so that the final volume is 5.0 ml. This solution is then analyzed on the flame photometer for copper and iron.

The filtrate from the copper and iron precipitation is treated as follows to determine manganese. Add 5 ml. of 8-hydroxyquinoline solution followed by 1 to 1 ammonium hydroxide to a pH of 7.1. Stir the solution thoroughly, cover with a watch glass, and allow to stand overnight. The precipitates are recovered and treated in exactly the same manner as described for copper and iron and used to determine manganese. Distilled water as a blank is carried through the same procedure.

Table I. Wave Lengths and Instrumental Conditions for Determining Trace Elements in Plant Material

Element	Wave Length, $M\mu$	Sensitivity	Concn. Range, P.P.M.	Slit Width, Mm.
Iron	372.0	5 turns clockwise	0-200	0.025
Manganese	403.3	5 turns clockwise	0-100	0.06
Copper	324.8	Maximum	0-10	0.04

Choice of Wave Lengths. Gilbert (77) lists the various wave lengths and detection limits for 46 elements which can be determined by flame photometry. Those listed for copper, iron, and manganese were checked with standard solutions, and the most sensitive in each case was chosen for this investigation. Table I shows the wave lengths and instrumental conditions which were used to analyze trace elements in plant materials.

Standard Curves. Standard calibration curves were prepared for the elements copper, iron, and manganese by diluting suitable aliquots of 2000-p.p.m. standard solutions and determining the relative emission energies as indicated on the transmittance scale of the flame photometer. The per cent transmittance readings were corrected for background with a distilled water blank. Standard curves were typical of those obtained for other ions and were almost straight lines.

Results and Discussion

Ion Interferences. The determination of copper was not affected by the presence of iron or manganese in the range of concentrations encountered in samples of plant ash. This verified work of Dean (4) who reported on these same elements in nonferrous alloys. Manganese can be measured quantitatively in the presence of iron but the reverse

is not true as manganese interferes with the iron determinations. This effect is illustrated by the data in Table II and is in agreement with the results of interference studies carried out by Dippel and Bricker (8) and Dean and Burger (5). These data indicate that a separation of manganese from iron is necessary if iron concentrations are desired.

Aluminum is used as a carrier precipitate in the procedure. After preliminary data indicated optimum amounts of aluminum for the precipitation, solutions containing this amount of aluminum were prepared and no interferences with copper, iron, or manganese were encountered. If details of the proposed procedure are followed, the maximum concentration of aluminum in the test solution will not exceed 400 p.p.m. The absence of interferences at this level of aluminum concentration verified the results of the work of Dean and Burger (5) who reported little interference of aluminum on iron when aluminum was present in amounts below 500 p.p.m.

Element Separation and Ashing Techniques. In order to investigate separation, ashing techniques, and recovery of elements, a synthetic plant solution was prepared as follows. These ion concentrations are averages for plant materials as reported by Beeson (2).

Element	Concentration, P.P.M.
Copper	10
Cobalt	2
Iron	400
Boron	25
Manganese	75
Calcium	20,000
Magnesium	2,500
Potassium	20,000
Sodium	1,500

The concentration of cobalt was increased by approximately a factor of 20 over the average values listed by Beeson. This was done in the hope of being able

to measure the concentration of this element in the presence of the others by flame photometry. However, this increased level of cobalt still was too low to be determined conveniently by flame techniques.

As was shown above, some separation of the ions in solution must be accomplished before a quantitative determination of the elements can be achieved by flame photometric methods. This could be either separation of manganese from iron and copper, or separation of iron from manganese and copper.

The data of Fleck and Ward (9), and Goto (72) indicated that iron and copper might be separated from manganese by precipitating with 8-hydroxyquinoline at a pH of 5.1. Scott and Mitchell (75) utilized the aluminum ion as a carrier precipitating agent in their analysis for trace elements in plants and soils. When the trace elements in the synthetic plant solution were separated by the 8-hydroxyquinoline method and ashed by digestion with concentrated sulfuric acid and 30% hydrogen peroxide, the results obtained for iron, copper, and manganese did not agree with the amounts known to be present. When the separation of the elements was carried out as before, and the ashing performed according to the method of Smith (76), recovery was much better and agreed well with the known concentrations. However, the ashing procedure of Smith, utilizing nitric and perchloric acids, is time consuming and dangerous. Therefore, dry ashing at two different temperatures, 640° and 850° C. was investigated. Results for recovery of trace elements from the synthetic plant solution are shown in Table III. These data indicate that the dry ashing technique at 850° C. gives slightly better results for iron and manganese than at 640° C., and equally good results for copper at 640° C. Attempts to place the precipitate from the 8-hydroxyquinoline treatment in solution with acid and determine the flame emission of the

Table II. Effect of Iron on Determination of Manganese and Manganese on Determination of Iron by Flame Photometry

Actual Concn., P.P.M.		Concn. of Mn, P.P.M.
Mn	Fe	
10	0	10
10	50	10
10	100	10.2
10	500	10.3
50	0	50
50	50	50.5
50	100	50.5
50	500	50.6
100	0	100
100	50	101.3
100	100	101.5
100	500	101.8

Actual Concn., P.P.M.		Concn. of Fe, P.P.M.
Fe	Mn	
50	0	50
50	10	50
50	50	50
50	100	75
50	200	100
100	0	100
100	10	105
100	50	115
100	100	130
100	200	155
500	0	500
500	10	500
500	50	500
500	100	500
500	200	540

Table III. Comparison of Recovery of Cu, Mn, and Fe from Dry Ashed Synthetic Plant Solutions

Sample	Copper		Iron		Manganese	
	Actual, P.P.M.	Found, P.P.M.	Actual, P.P.M.	Found, P.P.M.	Actual, P.P.M.	Found, P.P.M.
			640° C.			
Blank	0	0	0	0	0	0
Synthetic plant	10	9.75	400	325	75	65
	10	9.75	400	325	75	65
	10	9.75	400	325	75	65
			850° C.			
Blank	0	0	0	0	0	0
Synthetic plant	10	9.50	400	360	75	77.5
	10	9.50	400	360	75	77.5
	10	9.75	400	375	75	77.5

Table IV. Determination of Copper, Iron, and Manganese in Plant Material in Parts Per Million

Sample	AOAC Method			Flame Method (Dry Ash, 850° C. ^a)			Flame Method (Wet Ash) ^b
	1st detn.	2nd detn.	Av.	1st detn.	2nd detn.	Av.	1st detn.
COPPER							
Soybeans							
C ²⁸	28.8	25.0	26.9	22.5	24.8	23.7	26.8
C ²⁹	31.5	30.9	31.2	...	25.5	25.5	31.0
C ³⁰	35.0	27.7	31.4	37.7	29.5	33.6	33.2
Corn							
C ³¹	5.3	5.8	5.6	4.1	6.0	5.1	4.0
C ³²	4.8	3.1	4.0	3.0	3.5	3.3	3.5
C ³³	8.0	4.6	6.3	5.3	5.3	5.3	5.4
Wheat							
C ³⁷	6.3	5.9	6.1	...	7.2	7.2	15.0
C ³⁸	5.5	3.8	4.7	6.3	6.5	6.4	19.0
C ³⁹	9.0	8.0	8.5	10.8	6.5	8.6	9.8
Oats							
C ⁴⁴	36.3	36.2	36.3	32.3	32.8	32.6	2.5
C ⁴⁵	33.3	34.6	34.0	30.3	29.5	29.9	14.0
C ⁴⁷	31.5	32.2	31.9	37.5	25.0	31.2	16.5
Alfalfa							
C ⁴⁸	17.5	13.6	15.6	15.5	14.0	14.8	12.8
C ⁴⁹	17.0	13.9	15.5	17.5	18.0	17.8	14.0
C ⁵⁰	19.6	14.9	17.3	16.8	15.5	16.2	14.0
Synthetic plant	9.3	9.5	9.4	12.0	9.8	10.9	13.2
IRON							
Soybeans							
C ²⁸	82	89	85.5	110	92.5	101.3	125
C ²⁹	90	90	90.0	90	85.0	87.5	30
C ³⁰	88	82	85.0	80	85.0	82.5	107
Corn							
C ³¹	16	12	14.0	23.0	25	24.0	35
C ³²	16	16.2	16.1	25.5	25	25.3	21.7
C ³³	16	12	14.0	20.5	16.5	18.5	21.7
Wheat							
C ³⁷	43	30	36.5	42.5	40.0	41.3	50.0
C ³⁸	35	28	31.5	36.2	35.0	35.6	30.0
C ³⁹	30	25	27.5	32.5	30.0	31.2	20.0
Oats							
C ⁴⁴	63	69	66.0	61.3	62.5	61.9	46.3
C ⁴⁵	43	42	42.5	50.0	43.8	46.9	55.0
C ⁴⁷	43	46	44.5	47.5	43.8	45.6	50.0
Alfalfa							
C ⁴⁸	317	325	321	262	292	277	330
C ⁴⁹	240	240	240	205	218	212	285
C ⁵⁰	213	234	224	247	228	257	207
Synthetic plant	376	362	369	357	398	377	410
MANGANESE							
Soybeans							
C ²⁸	27.0	26.5	26.8	24.5	30.0	27.3	5.0
C ²⁹	27.8	30.0	28.9	25.0	31.3	28.1	2.5
C ³⁰	27.5	28.0	27.8	28.7	30.0	29.4	2.5
Corn							
C ³¹	4.5	4.4	4.5	4.0	4.3	4.2	1.7
C ³²	4.0	3.8	3.9	4.0	4.3	4.2	1.8
C ³³	4.5	3.8	4.2	4.0	4.3	4.2	0.0
Wheat							
C ³⁷	47.5	45.0	46.3	37.0	38.8	37.9	2.5
C ³⁸	36.3	38.8	37.6	30.0	36.2	33.1	2.5
C ³⁹	36.3	37.5	36.9	33.0	38.8	35.9	1.7
Oats							
C ⁴⁴	39.5	45.0	42.3	40.5	42.0	41.3	2.5
C ⁴⁵	37.5	40.0	38.8	33.0	38.8	35.9	2.5
C ⁴⁷	37.7	45.0	41.4	35.0	38.8	36.9	2.5
Alfalfa							
C ⁴⁸	72.5	73.0	73.0	41.3	36.5	54.9	8.8
C ⁴⁹	125.0	120.0	122.5	90.0	109.0	99.5	22.5
C ⁵⁰	96.0	106.0	101.0	72.0	92.2	83.1	12.5
Synthetic plant	71.5	75.0	73.3	75.0	78.0	76.5	5.0

^a Precipitated at pH 7.0 to 7.2 in determination of manganese.

^b Precipitated at pH 5.1 in determination of copper and iron and at pH 5.9 to 6.1 in determination of manganese.

elements were unsuccessful due to very high background readings.

Casto (3) indicated that an electrolytic process had been used on the Manhattan Project, as a means of separating trace elements from a uranium solution. An attempt was made to utilize this technique to separate the trace elements in a solution of copper, manganese, and boron. However, the results obtained were very low compared with known concentrations.

Choice of Plant Samples. After the analytical procedure had been developed through utilization of a synthetic plant solution, it appeared advisable to check the procedure with representative samples of plant material. In the selection of plant samples including grain, grass, and legumes, there was a wide range of concentration levels of copper, iron, and manganese. The samples which were analyzed in this investigation were all grown at the Kansas Agricultural Experiment Field near Columbus, Kan.

Types of Analyses. The plant materials utilized in this investigation were analyzed by three methods: the Association of Official Agricultural Chemists (AOAC) procedures (7), the flame-photometric method after wet ashing the 8-hydroxyquinoline precipitates, and the flame photometric process after dry ashing the 8-hydroxyquinoline precipitates. The results of these three types of analyses on plant materials and synthetic plant solution are presented in Table IV. The values shown for manganese by the wet ashing procedure were obtained on precipitates which had been formed at pH of 5.9 to 6.1. The dry ashing values were obtained after precipitation of the manganese at a pH of 7.0 to 7.2. Therefore, the results of the wet ashing technique cannot be compared with the AOAC method or dry ashing techniques for the determination of manganese. However, the wet ashing results indicate that precipitation of manganese was not complete at a pH of 5.9 to 6.1.

In considering the data presented in Table IV, some variations exist when comparing the flame method with the AOAC method. The variations are slightly more pronounced in the determination of iron than for copper and manganese. No attempt was made to determine why these variations exist or to correlate the variations to the plant materials under consideration.

To compare the flame photometric method with the AOAC method statistically, a series of 10 analyses was conducted on one sample. The plant sample chosen for this analysis was carbon-47, which appeared to have approximately average concentrations of copper, iron, and manganese when compared with the other samples analyzed. The results of these series of

analyses are presented in Table V. The flame method would probably give slightly lower results for copper and manganese than the AOAC method, while iron is slightly higher. The results by the flame method are as precise as those by the AOAC method.

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Table V. Comparison of Results on a Series of 10 Analyses on Same Sample

	Copper, P.P.M.		Iron, P.P.M.		Manganese, P.P.M.	
	AOAC	Flame photometric (dry ash)	AOAC	Flame photometric (dry ash)	AOAC	Flame photometric (dry ash)
	31.5	37.5	43.0	47.5	37.7	35.0
	32.2	25.0	46.0	43.8	45.0	38.8
	37.8	29.5	37.0	40.0	39.0	38.0
	37.8	31.0	37.0	40.0	37.5	38.0
	39.0	31.2	38.0	45.5	39.0	37.0
	39.5	34.0	36.0	40.0	40.0	37.5
	40.2	32.2	40.0	36.2	39.0	35.0
	38.2	33.0	40.0	40.0	41.0	38.8
	42.0	37.0	42.0	43.8	41.0	37.5
	37.2	32.2	34.0	43.8	40.0	40.0
Mean	37.5	32.2	39.3	42.1	39.9	37.6
S.D.	3.21	3.60	3.62	3.36	2.14	1.60

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HERBICIDE LEACHING

Mobility of *N*-1-Naphthylphthalamic Acid (Alanap-1) in Soil

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N-1-Naphthylphthalamic acid, a pre-emergence herbicide, shows moderate mobility in soil in spite of its very low water solubility. Although some mobility is desirable to assure even distribution of the chemical in the weed-producing soil layer, it should be controlled to protect semisensitive crops and to prolong residual herbicidal effect. Three ways of controlling the mobility of the herbicide have been found.

MOBILITY IN SOIL is an important factor in the performance of a pre-emergence herbicide. Some mobility in soil is desirable, to assure distribution throughout the weed-producing soil layer. It is advantageous, however, to limit the downward movement of the chemical in soil in heavy rainfall areas, as this prolongs the residual herbicidal effect and decreases chances of injuring deep-planted seeds of semisensitive crops.

The mobility of herbicides in soil varies greatly. Studies of the leaching characteristics of several herbicides were reported by Ogle and Warren (7). More

recently, the strong adsorption of 3-amino-1,2,4-triazole by soil particles was reported (4). In general, water-soluble herbicides tend to move readily with soil water, while most water-insoluble herbicides exhibit low mobility in soil. Although *N*-1-naphthylphthalamic acid has a very low water solubility, moderate mobility in soil was suspected from field performance data in 1952.

The Alanap herbicides (*N*-1-naphthylphthalamic acid derivatives) appear to give selective herbicidal action for two reasons: Some crops are only slightly susceptible—for example, most cucur-

bits—and crop seeds planted 1 inch or more below the soil surface are somewhat protected from chemicals applied to the surface; while weed seeds, which usually germinate in the top 1/4 inch of soil, are subjected to much higher concentrations of the chemical during the germination period. Injury to crops, such as cotton and beans, treated with *N*-1-naphthylphthalamic acid has varied from negligible to severe in field trials under different conditions. Differences in the amount of *N*-1-naphthylphthalamic acid leached to crop-seed depth in different tests could account for such variations.