

Micro-determination of Zinc and Copper in a Single Digest of Small Samples of Plant Material

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WHEN only very small samples of plant material are available, it may be impossible to carry out multiple digestions. In the determination of zinc with dithizone it is necessary to remove interfering copper, and a method is proposed in which the separated copper is conserved and determined, a single digest of plant material thus sufficing for both determinations.

METHOD

The determination of zinc is based on the method of Cowling and Miller¹ as modified by Verdier, Steyn and Eve²; the determination of copper is based on the recommended method of the Society for Analytical Chemistry,³ in which sodium diethyldithiocarbamate is used.

REAGENTS—

All reagents must be of analytical-reagent grade.

Nitric acid, sp.gr. 1.42.

Sulphuric acid, sp.gr. 1.84.

Perchloric acid, 60 per cent. w/v.

Ammonium hydroxide, approximately 3.5 N—Dilute 200 ml of ammonia solution, sp.gr. 0.88, to 1 litre with water.

Hydrochloric acid, N and 0.02 N.

Carbon tetrachloride—Redistilled.

Dithizone solution, 0.01 per cent. w/v—Dissolve 0.1 g of dithizone (diphenylthiocarbazon) in 250 ml of carbon tetrachloride, set the solution aside in a refrigerator for a minimum of 12 hours, filter it and dilute it to 1 litre with carbon tetrachloride. Store the solution in a dark-coloured bottle in a refrigerator.

Solution A—Dissolve 52.5 g of nitric acid in water and add ammonia solution, sp.gr. 0.88, until pH 9 is reached, using narrow-range test papers to estimate the pH. Add 75 ml of N ammonium hydroxide and dilute the solution to 1 litre with water.

Solution B—Dissolve 46.6 g of citric acid in water, and add 50 ml of ammonia solution, sp.gr. 0.88, and dilute the mixture to 1 litre with water. (The pH of this solution should be about 9; if it is not, adjust it to this value with ammonia solution.)

Sodium diethyldithiocarbamate—Dissolve 1 g of the solid in 100 ml of water; store the solution in the dark in a refrigerator. Filter it before use; discard it after 7 days.

EDTA - citrate solution—Dissolve 17.28 g of citric acid in water and neutralise it with ammonia solution, sp.gr. 0.88. Add 5 g of EDTA (disodium salt) and dilute the mixture to 1 litre with water.

Standard solutions of zinc and copper—Prepare separate stock solutions of the sulphates to contain 100 µg of the metals per ml, acidifying each solution with 1 ml of sulphuric acid per litre. Dilute the stock solutions as required to give standard solutions containing 10 µg of metal per ml.

PRECAUTIONS AGAINST CONTAMINATION—

Rigid precautions must be taken at every stage of preparation of the plant material. At harvesting, soil contamination must be avoided; galvanised trays or similar oven fittings must be excluded, and stringent precautions must be taken to prevent contamination from cosmetics.

All glassware must be scrupulously clean, and must be checked by rinsing with dithizone solution until there is no change in the clear green colour of the solution.

PROCEDURE—

Weigh 0.5 g of dried plant material into a 50-ml Kjeldahl flask, and digest the sample with 10 ml of nitric acid, 1 ml of sulphuric acid and 2 ml of perchloric acid, added in the order given. Heat the mixture until a clear digest is obtained, and then continue heating to fuming for another 20 minutes. Cool and wash the contents of the Kjeldahl flask into a 50-ml calibrated flask with about 30 ml of water. Neutralise the solution to methyl red with 3.5 N ammonium hydroxide, add 2 ml of N hydrochloric acid and dilute the solution to the mark.

Transfer a suitable aliquot containing not more than 20 μg of zinc or copper to a 100-ml separating funnel. Add 40 ml of solution *A* and 10 ml of dithizone solution. Shake the funnel for 2 minutes. Run off the lower (carbon tetrachloride) layer into a second separating funnel, and extract the aqueous layer with at least two successive 5-ml portions of dithizone solution, continuing until the extract is a clear green. Reject the aqueous phase.

Add 50 ml of 0.02 *N* hydrochloric acid to the combined carbon tetrachloride extracts, and shake the mixture for 2 minutes. The zinc passes into the aqueous phase, leaving the copper in the organic phase, which is transferred to a 50-ml Kjeldahl flask and retained for the determination of copper. Extract the aqueous phase twice with consecutive 5-ml portions of carbon tetrachloride, adding these to the contents of the Kjeldahl flask.

To the aqueous phase in the separating funnel, add 15 ml of solution *B* and 10 ml of dithizone solution, and shake the funnel for 2 minutes. Run off the carbon tetrachloride phase and extract the aqueous phase with an additional 5 ml of dithizone solution. Combine the carbon tetrachloride extracts and add 35 ml of carbon tetrachloride (or other suitable volume to dilute the coloured complex appropriately). Read the optical density at 535 $m\mu$ in a spectrophotometer with carbon tetrachloride as the reference solution.

Evaporate the carbon tetrachloride solution of the copper - dithizone complex to dryness. Add two drops of sulphuric acid and 5 ml of nitric acid. Heat the mixture to fuming and continue heating for 10 minutes. Cool, add 10 ml of EDTA - citrate solution and two drops of thymol blue indicator. Add 3.5 *N* ammonium hydroxide until the colour is green (NOT blue), and then add 1 ml of sodium diethyldithiocarbamate and 10 ml of carbon tetrachloride. Shake the flask for 2 minutes and run off the carbon tetrachloride layer into a cuvette and read the optical density at 436 $m\mu$.

PREPARATION OF CALIBRATION GRAPHS—

A blank determination with the same amounts of all reagents is made, and is used together with similar determinations for mixtures of the zinc and copper standards containing 10, 20 and 30 μg of each to obtain calibration curves for both metals. By adhering rigidly to the procedure and by using exactly the same amounts of all reagents, the calibration curve obviates the necessity for elaborate purification procedures for all reagents.

The blank values indicated by the calibration curves show the degree of reagent contamination with copper and zinc. If this approaches an unacceptable level, the reagents should be purified by the usual procedures.³ A fresh calibration curve must be prepared for each set of determinations, and whenever a new batch of any reagent is used.

Direct exposure of the zinc - dithizone and copper - diethyldithiocarbamate complexes to sunlight is to be avoided. Both are stable, however, to diffuse daylight for short periods, and accurate results can be obtained provided that there is no undue delay in completing the determinations.

RESULTS

Results are given in Table I for 0.5-g samples of oven-dried (105° C) material taken from 6-week-old plants grown at Wellesbourne in a sandy loam of the Newport series.

TABLE I
RESULTS FOR COPPER AND ZINC FOUND IN OVEN-DRIED PLANT MATERIAL

Plant material	Found, p.p.m.		Added,* p.p.m.		Found, p.p.m.		Recovery, per cent.	
	zinc	copper	zinc	copper	zinc	copper	zinc	copper
Red beet	11.2	3.8	20	20	32.8	25.8	108	110
Cabbage	10.2	2.0	20	20	30.2	23.0	100	105
Carrots	14.0	11.8	20	20	34.2	32.4	101	103
Lettuce	17.4	6.0	20	20	36.6	25.8	96	99
Onion	18.0	6.2	20	20	36.8	26.6	94	102

* Added as solutions of the respective sulphates to aliquots of the digest.

REFERENCES

1. Cowling, H., and Miller, E. J., *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 145.
2. Verdier, E. T., Steyn, W. J. A., and Eve, D. J., *J. Agric. Food Chem.*, 1957, **5**, 354.
3. Society for Analytical Chemistry, "Determination of Trace Elements with Special Reference to Fertilisers and Feeding Stuffs," W. Heffer & Sons Ltd., Cambridge, 1963.

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