

DETERMINATION OF COPPER AND ZINC IN SUGAR-CANE LEAVES BY ATOMIC ABSORPTION

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Introduction

Since the introduction by Walsh¹² in 1955 of atomic absorption spectrophotometry as an analytical method, it has found application in many fields as a means of rapid analysis of certain metallic elements at very low concentrations. The analysis of agricultural materials, i.e., leaves, soil extracts and fertilizers is one sphere in which atomic absorption has been applied by several workers^{1, 2, 4, 5} for the analysis of Na, Mg, Ca, K as well as Cu and Zn. This method has a number of advantages over the usual colorimetric and flame photometric determinations of these elements; (a) it is relatively free from interference by other elements in the sample, (b) it has a higher sensitivity than the flame photometric methods and (c) it is much faster than the usual colorimetric methods.

Atomic absorption spectrophotometry is based on the principle that free atoms of an element can absorb the characteristic radiation, known as resonance radiation, of that specific element. This phenomenon is well known in producing the Fraunhofer lines in the solar spectrum. The amount of light absorbed by the atoms of the element is proportional to the number of atoms through which the light beam is passed, and therefore proportional to the concentration of the element in the sample, provided that the sample is atomised at a constant rate.

The atomic absorption spectrophotometer consists essentially of a light source producing the resonance radiation of the element, a flame for atomizing the sample, a means of isolating the resonance radiation from unwanted background and non-resonance radiation, and a photo-electric detector to measure the intensity of the light after it has passed through the flame. The operation of the instrument is closely analogous to a spectrophotometer in which the absorption cell has been replaced by the flame, and the light source usually emitting continuous radiation by a lamp emitting the resonance radiation of the element. A hollow cathode lamp operating at a low current is used as a light source for all but the alkali elements where discharge lamps can be used. The current through the lamp has to be kept constant in order to avoid changes in intensity and sensitivity when analysing a series of standards or samples. It is obvious that a long absorbing path would increase the sensitivity, that is, the percentage of incident light absorbed per part per million of the element in the sample. The burner head is therefore designed to provide a long thin flame as opposed to the small confined flame required in flame emission spectrophotometry.

As the number of atoms in the flame is not only dependent on the concentration of the element in the sample but also on the rate at which the sample is sprayed into the flame, the rate of sample uptake must also be closely controlled to prevent changes in

sensitivity. This can be accomplished by stabilizing the flow of air aspirating the sample into the flame. Spectrometers for isolating the resonance radiation of the elements have degrees of sophistication varying from simple filter separation to double beam spectrophotometers in which the light beam from the hollow cathode lamp is split into two separate beams, one of which is used as a continuous monitor of the intensity of the hollow cathode lamp, and the other one passed through the atom cloud in the flame.

As the atomic absorption method, like flame photometric and colorimetric methods, does not provide an absolute measurement of the concentration of an element in a sample, the use of standard solutions for comparison is necessary, and it is therefore essential that no changes in sensitivity occur while determining a series of standards and unknown samples. Variations in air flow, air pressure and viscosity of the sample alter the rate of sample uptake which influences the density of the cloud of atoms in the flame. Variations in fuel flow to the flame change the temperature and character of the flame which may in turn affect the degree of breakdown of the sample. Fluctuations of the current through the hollow cathode lamp affect the amount of absorbable light passing through the flame, altering the percentage of incident light absorbed for a specific concentration. All these variables except the emission from the hollow cathode lamp are fairly easy to control if the necessary care is taken while a series of determinations is being carried out.

The atomic absorption spectrophotometer used for this work was the Perkin Elmer model 303, with a burner regulator providing control of air and fuel flow and standard burner using an air-acetylene mixture. The instrument was obtained in order to facilitate the routine analysis of Zn and Cu in plant tissue for fertilizer advisory purposes.

Currently leaf samples are routinely analysed for Na, K, Mg, Ca, P and N after being digested with sulphuric acid in the presence of selenium as a catalyst. Zinc and Cu are determined colorimetrically with diphenylthiocarbazone (dithizone). This employs a separate sub-sample digested with a mixture of nitric and sulphuric acids, as the selenium used in the digestion for the macro-elements also forms a diphenylthiocarbazone complex interfering with the Cu and Zn determinations. For both methods of digestion 2.5 gm. of leaf sample are taken and made up to 100 ml. after digestion, thus diluting the constituents in the leaf 40 times. With this dilution the extracts prepared with sulphuric acid and selenium, and those prepared with nitric and sulphuric acids contain 20 per cent and 8 per cent v/v sulphuric acid, respectively.

The requirements for a procedure to replace the colorimetric method for Cu and Zn would be (i) an accuracy at least equal to or better than the colori-

metric method and (ii) a higher speed and greater simplicity of analysis plus a minimum of sample preparation. It would also be advantageous if the determination could be carried out on the same digest as the macro-elements and if both Cu and Zn could be determined on the same solution.

Preliminary Investigation

From the work of Bradfield and Spincer⁴, it seemed hopeful that both Zn and Cu could be determined directly on the selenium-sulphuric acid digest, thus eliminating a separate digestion for the trace elements. Work carried out in establishing the optimum operating conditions given in Table I showed that adequate sensitivity and accuracy could be obtained for Zn, confirming results reported by David⁵ and Bradfield⁸,

TABLE I
Standard Operating Conditions

PARAMETERS	ZINC		COPPER	
	Aqueous solutions	MIBK extracts	Aqueous solutions	MIBK extracts
Air pressure . . .	30 p.s.i.	25 p.s.i.	30 p.s.i.	25 p.s.i.
Air flow (litres/min.) . . .	5.0	4.7	5.0	4.7
Air flow gauge setting . . .	2.7	2.5	2.7	2.5
Auxiliary air flow (litres/min.) . . .	10.6	6.3	10.6	6.3
Auxiliary air flow gauge setting . . .	6.3	4.0	6.3	4.0
Total air flow gauge setting . . .	9	6.5	9	6.5
Acetylene flow (litres/min.) . . .	3.0	1.6	3.0	1.6
Acetylene flow gauge setting . . .	9	4.5	9	4.5
Sample consumption (ml./min.) . . .	2.46	3.0	2.46	3.0
Scale expansion . . .	2x	1x	5x	2x
Wavelength . . .	2138A		3247A	
Slit setting . . .	5		4	
Slit width . . .	3 mm. (20A)		1 mm. (7A)	
Lamp operating current . . .	9 mA		10 mA	

The method suggested by Wilson¹³, where the detection limit of a method is expressed as a multiple of the standard error of the background, was used to determine the detection limits for Cu and Zn. The standard error of the background was found to be 0.02 ppm. for Zn and 0.014 ppm. for Cu. This gives limits of detection for Zn and Cu respectively of 0.06 ppm. and 0.04 ppm. in solution, and 2.4 ppm. and 1.6 ppm. in the undiluted leaf material. As the critical limit for the Cu content in the leaf is taken for fertilizer advisory purposes as 3 ppm., the sensitivity for Cu is inadequate to ensure accurate determinations at the concentrations near this limit. Bradfield and Spincer⁴ obtained satisfactory results because the Cu content in their samples varied from 12 to 16 ppm. in the original plant material, which is appreciably higher than the usual concentrations of 2 to 10 ppm. Cu occurring in sugarcane leaves.

Determination of Cu and Zn after Concentration by Organic Solvent Extraction

The difficulty experienced in the determination of low concentrations of trace elements has been overcome by Allan¹, and Strasheim, Eve and Fourie⁹ by concentration of the trace elements through their extraction with an organic solvent, and also by Strelow¹¹, who combined organic solvent extraction with a cation exchange separation. As the atomic absorption method is free from interference by Fe as opposed to some emission spectrometric methods, it is unnecessary to include an ion exchange separation of Fe from the other trace elements. Therefore only the possibility of the simultaneous extraction of Cu and Zn with an organic solvent was investigated.

Ammonium pyrrolidine-dithiocarbamate was suggested as a complexing agent by Malissa and Schöffman⁶, who pointed out that both Zn and Cu formed a complex with this reagent at pH levels from 2 to 9, while selenium formed only a weak complex with it. Similar reagents were used for the concentration of trace elements by several workers^{1, 8, 10, 11} and it was hoped that it would be possible by correct choice of pH to eliminate interference of the complexing agent by selenium.

A quantity of ammonium pyrrolidine-dithiocarbamate was prepared by the method of Malissa and Schoffman⁶ from redistilled and purified reagents. Unfortunately the excess of selenium in the leaf samples digested with selenium and sulphuric acid was so great that it was impossible to eliminate the interference of selenium by choice of pH. Other methods of removing the selenium from solution by oxidation, or precipitation as elemental selenium, also proved unsuccessful, and the separate digestion with nitric and sulphuric acids has therefore to be used.

The effect of pH on the extraction is shown for three different concentrations of Zn and Cu in Figure 1, and demonstrates that the extraction of Cu varies very little with pH over the range pH 3.5 to pH 5.8, but that a sharp decrease in the efficiency of the Zn extraction occurs at pH 4.7. This means that the extraction should be carried out at a pH between 5.5 and 5.8 which is contrary to the report of Allan¹, who obtained 100 per cent extraction of Zn from pH 2.5 to 5.0.

Procedure

Reagents

1. A saturated solution of sodium acetate purified by extraction with dithizone and CCl₄.
2. A 1 per cent aqueous solution of ammonium pyrrolidine-dithiocarbamate.
3. Methyl isobutyl ketone purified if necessary by extracting with 6N HCl.

Method

A leaf sample of 2.5 gm. is weighed out and digested with 5 ml. H₂SO₄ + 15 ml. HNO₃ until, when white fumes of H₂SO₄ are evolved, a clear solution is obtained. Additional portions of HNO₃ are added if necessary to obtain a clear solution. After cooling, the leaf extract is made up to 100 ml. A 15 ml. aliquot

is then transferred to a 50 ml. separating funnel after which 15 ml. buffer solution, 1 ml. 1 per cent ammonium pyrrolidine-dithiocarbamate, and 4 ml. methyl isobutyl ketone are added. The separating funnel is shaken mechanically for three minutes and the organic phase run into a thimble. A series of standards containing 0.1 to 1.0 ppm. Zn and 0.1 to 0.4 ppm. Cu in 1.1 N H_2SO_4 is made up and extracted in the same way. The solutions and standards are aspirated with the instrument settings given in Table I, the zero of the instrument being set with pure methyl isobutyl ketone. The percentage absorption for each sample and standard is measured, and the Cu and Zn in the unknown samples are calculated from the working curve obtained from the standards.

The coefficient of variation for duplicate readings, taken over 20 extractions of the same sample was 13 per cent for a concentration of 0.4 ppm. Zn, and 10

per cent for Cu at a level of 0.1 ppm. A comparison between the results obtained for five leaf samples with the colorimetric method and the atomic absorption method following the extraction procedure is given in Table II.

TABLE II
Comparison between Results obtained with the Dithizone Colorimetric and Atomic Absorption Spectrophotometric Method after Concentration

SAMPLE NO.	ZINC (ppm.)		COPPER (ppm.)	
	Atomic absorption	Dithizone	Atomic absorption	Dithizone
1	18	22	7.8	7.0
2	20	23	6.0	6.5
3	25	22	8.3	7.5
4	20	19	9.4	8.1

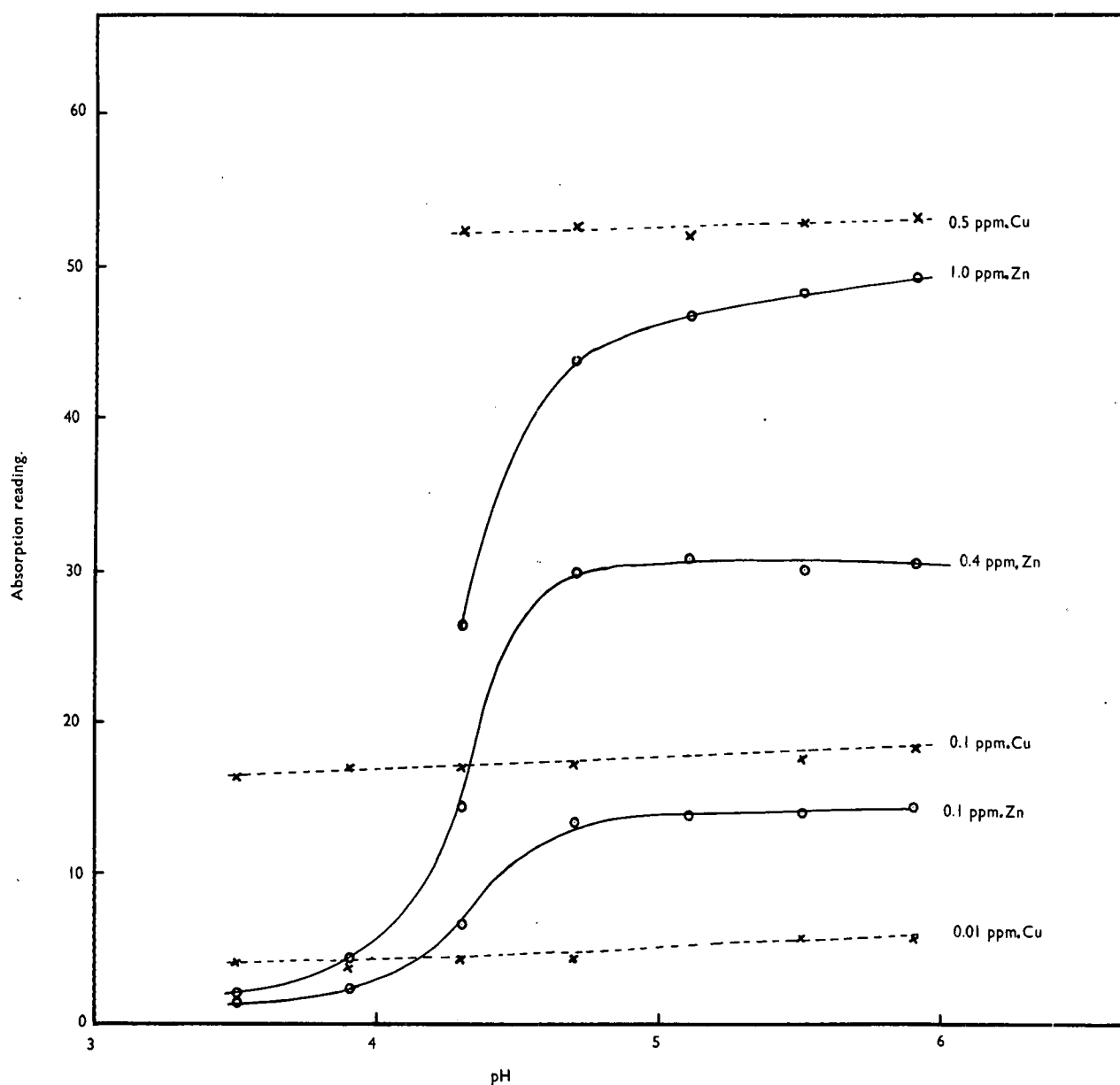


Figure 1. Effect of pH on extraction.

Direct Determination of Cu and Zn on the Leaf Extract

A direct determination of Cu and Zn on leaf samples digested with nitric and sulphuric acids and diluted 1:10 instead of 1:40 was undertaken, but difficulty was experienced due to blocking of the flame slit by the high concentration of solids or sulphuric acid in solution. When samples were digested with a mixture of nitric and perchloric acids and diluted 1:10, the burner slit no longer became blocked, showing that the previous blocking was not due solely to the high solids content of the samples, but was aggravated by the sulphuric acid. As the digestion of plant material with nitric and perchloric acids may be dangerous, this method is unsuitable for routine use, and it is therefore necessary to add a small quantity of sulphuric acid to the sample before ashing according to the method of Piper⁷. It was found that if the sulphuric acid used in the digestion was limited to approximately 8 per cent in the final solution, blocking

of the burner slit was lessened to such an extent that it was no longer troublesome. No interference with the absorption of Cu and Zn by Na, K, Ca, Mg or phosphate at the levels occurring in the leaf extracts was found, thus confirming the results obtained by Allan¹ and David⁵.

The effect of different acids and acid concentrations on the readings obtained for Zn and Cu was investigated. It was found that up to 10 per cent hydrochloric acid and 8 per cent nitric acid had no effect on the readings, while sulphuric acid had a marked depressing effect at the higher concentrations of Cu and Zn as shown in Figures 2 and 3. At lower concentrations an enhancement of the Zn readings was observed, most probably due to contamination of the sulphuric acid with Zn. Satisfactory results were obtained when 8 per cent sulphuric acid was added to the standards, leaf samples being analysed according to the following procedure.

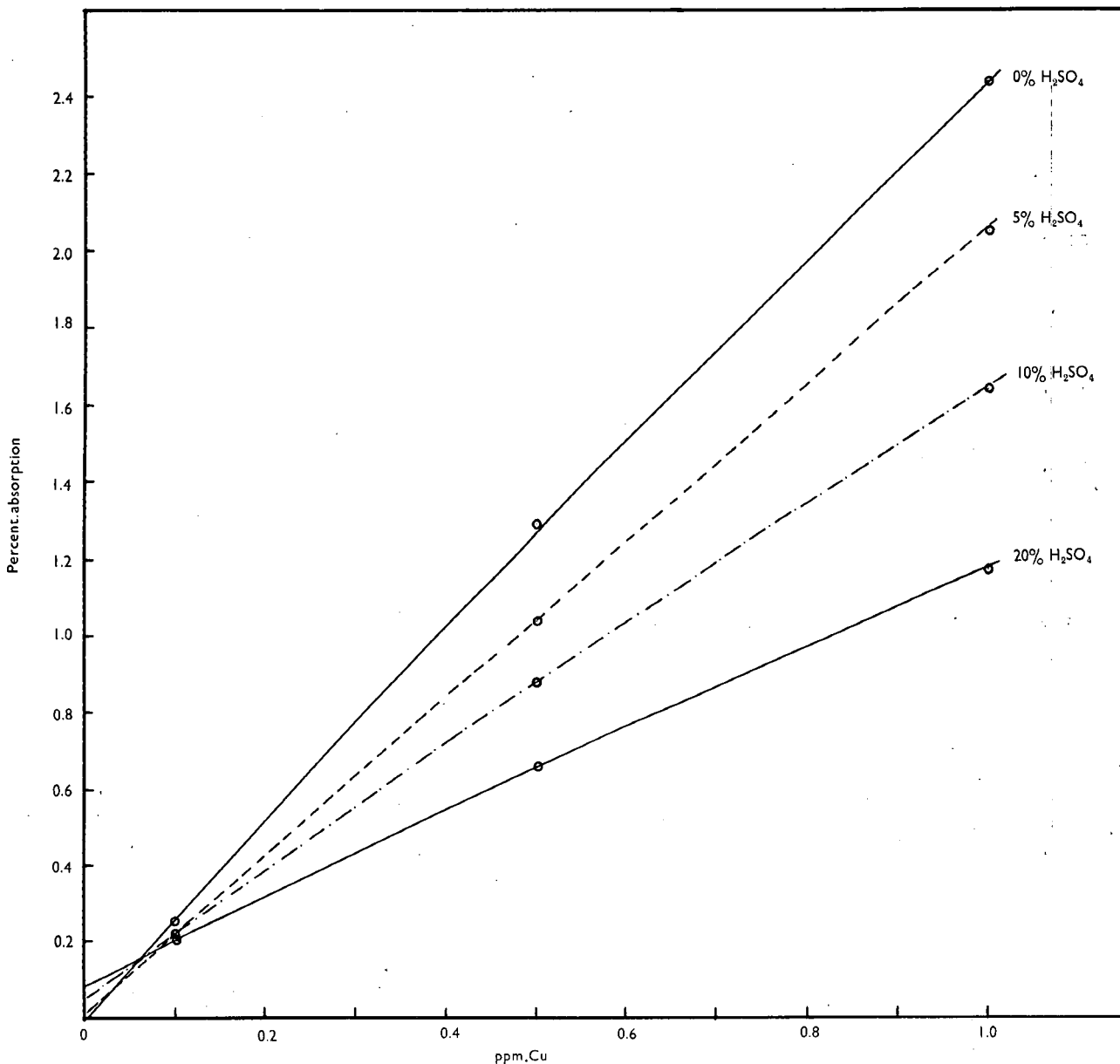


Fig. 2. Effect of different acid concentrations on Cu absorption.

Procedure

A sub-sample of 2.5 gm. is taken from the ground oven dried leaf sample and digested with H_2SO_4 : HClO_4 : HNO_3 in the ratio 2 : 3 : 20 ml. With proper control of the heating rate it is unnecessary to add more HNO_3 to obtain a clear extract. After adding 5 ml. H_2O the extract is transferred without filtering to a 25 ml. volumetric flask and made up to volume. The solutions are left overnight to allow the insoluble residue of silica to settle out completely. When aspirating, the sample tube of the burner is put directly into the volumetric flask without disturbing the sediment. This reduces handling of the sample considerably thereby saving time and lessening the chances of contamination. Standards containing 0.4 to 4 ppm. Zn and 0.04 to 1.5 ppm. Cu in 8 per cent H_2SO_4 are prepared, and samples and standards are aspirated

with the operating conditions shown in Table I. The zero of the instrument is set with distilled water and duplicate readings of per cent absorption for each sample are obtained by running through the series of samples and standards twice. In this way any drift in the instrument can be detected.

Results

Comparative figures for the results obtained from different digestions of six leaf samples by a method of addition not described, and direct determination, both with the atomic absorption spectrophotometer, and the dithizone colorimetric method are shown in Table III. The recovery of different amounts of Zn and Cu added to three leaf samples is given in Table IV, while Table V shows the reproducibility of the method as determined from a number of readings on each of 21 digestions of a single leaf sample.

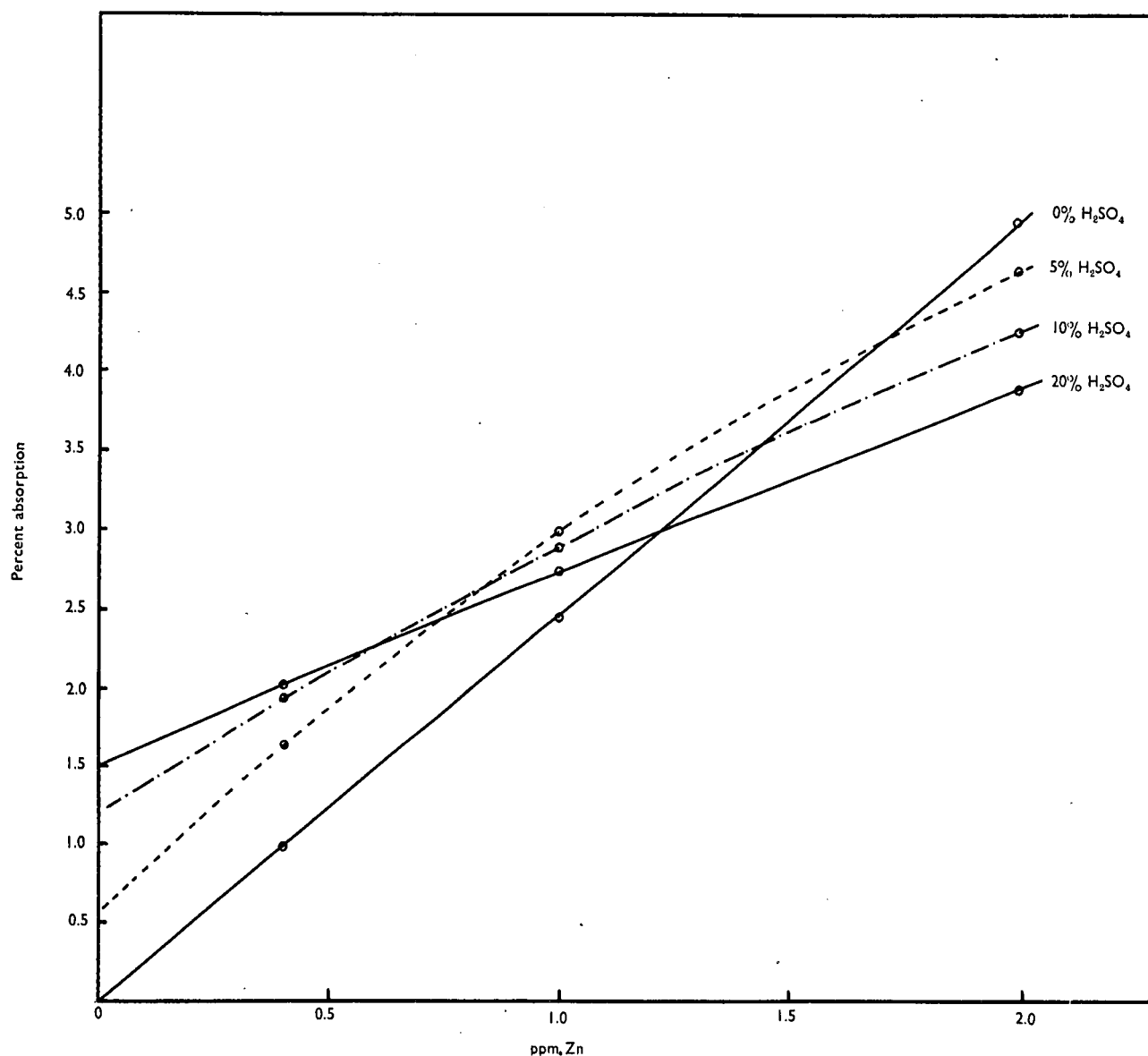


Figure 3. Effect of different acid concentrations on Zn absorption.

TABLE III
Comparison of Results obtained with the Direct Determination on Leaf Extracts by Atomic Absorption and Dithizone

Sample No.	ZINC (ppm.)							COPPER (ppm.)						
	Atomic absorption				Dithizone			Atomic absorption				Dithizone		
	Method of addition	Direct determination						Method of addition	Direct determination					
C1	—	8.1	7.6	8.6	9.5	9.5	11.5	—	4.6	4.5	4.0	4.5	5.0	5.0
C2	28.8	20.2	22.0	19.2	21.5	22.5	22.0	6.0	5.5	5.0	5.5	5.0	5.0	5.0
C3	—	8.8	7.8	7.8	7.5	8.0	8.0	—	6.0	6.5	6.1	7.0	7.0	7.0
C4	15.4	12.0	9.8	9.6	10.5	12.5	11.0	7.2	4.0	4.2	4.4	3.0	3.5	4.0
C5	26.2	19.2	17.2	18.2	19.0	19.0	19.0	8.2	6.4	6.2	6.4	7.0	6.5	7.0
C6	—	17.8	17.6	17.8	17.0	19.0	18.5	—	7.8	7.7	8.1	9.0	8.0	7.0

TABLE IV
Recovery by Atomic Absorption of Zinc and Copper added to Leaf Samples

Sample No.	ZINC (ppm.)				COPPER (ppm.)			
	Determined	Added	Found	Recovered	Determined	Added	Found	Recovered
C2	19.2	4	22.4	3.2	5.2	2	6.4	1.2
		8	26.5	7.3		4	8.2	3.0
		12	30.2	11.0		8	12.8	7.6
C4	9.1	4	13.1	4.0	4.5	2	6.5	2.0
		8	17.0	7.9		4	7.9	3.4
		12	23.1	13.1		8	12.7	8.2
C5	18.0	4	21.4	3.4	6.5	2	8.7	2.2
		8	27.5	9.5		4	11.0	4.5
		12	30.1	12.1		8	13.5	7.0

TABLE V
Reproducibility of Method

	ZINC	COPPER
Number of digestions	21	21
Number of readings on each digestion .	4	3
Contribution to standard error by digestion	0.592	0.191
Coefficient of variation of digestion	4.5%	4.8%
Contribution to standard error by reading errors	0.643	0.217
Coefficient of variation of readings	4.9%	5.2%
Standard error of method (duplicate readings on one digestion)	0.745	0.256
Coefficient of variation of method	5.7%	6.1%

This method has proved to be extremely rapid and it is possible to determine up to 30 samples per hour for a single element, which is appreciably more than can be handled when using an extraction method.

Discussion and Conclusion

From Table III it is evident that the accuracy of the atomic absorption method is not as high as expected, the results tending to be slightly lower than those obtained by the dithizone method. This is confirmed by the results in Table IV for the recovery of added amounts of Zn and Cu, which also indicate a bias towards lower values. This inaccuracy is possibly caused by the high solids content of the plant samples which might increase the viscosity of the sample, and therefore decrease the rate of sample uptake. It was not possible to determine the effect of high concentrations of solids in the sample, due to Cu and Zn contamination in the reagents used to prepare synthetic plant samples.

The results in Table III obtained with the method of addition, are much higher than those obtained by the dithizone method, and the direct determination with atomic absorption. This is due to a slight nonlinearity of the working curve. This nonlinearity is more pronounced for Zn than for Cu (see Figures 2 and 3) and explains why the results for Cu compared to those for Zn are closer to the results obtained by the other two methods.

As the extraction procedure with ammonium pyrrolidine-dithiocarbamate is more laborious, and less reproducible than the direct determination, the latter method is to be preferred unless the concentrations of Zn and Cu are very much lower than those usually occurring in sugarcane. The direct determination has the advantage over both the colorimetric and extraction methods, in that it is unnecessary to use specially purified reagents, as the standards contain the same quantity of sulphuric acid as the samples. The risk of contamination of the sample is also considerably lessened by reduced handling of the sample.

It is felt that the advantages of the direct determination of Cu and Zn by atomic absorption outweigh the somewhat lower accuracy of this procedure compared to the dithizone colorimetric method, and that it is sufficiently accurate to find application in the routine determination of Cu and Zn in sugarcane leaves.

Summary

Two methods for the analysis of Cu and Zn in sugarcane leaves by atomic-absorption spectrophotometry are described, and compared with the diphenylthiocarbazone (dithizone) colorimetric method. In the first procedure the Cu and Zn are concentrated by extraction as an ammonium pyrrolidine-dithiocarbamate complex in an organic solvent, while in the second the Cu and Zn are determined directly on the leaf digest.

The direct determination is much faster and more reproducible than the extraction procedure, and is to be preferred except when very low concentrations of Cu and Zn are encountered.

Although the accuracy of the direct determination by atomic-absorption is somewhat less than can be obtained by the dithizone method it is quite accurate enough for all practical purposes.

References

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Dr. Sumner: Were the hollow cathode lamps used of the sealed type?

Mr. du Preez: The C.S.I.R. advised us to use locally manufactured lamps that could be regenerated but we are having so much trouble with them that we are considering the use of sealed non-regeneratable lamps.

Dr. Matic: The atomic absorption method suffers from the disadvantage that the warming up of the lamps takes a long time. Has polarography been considered as a method for the simultaneous determination of copper and zinc? The half-wave potentials of these two metals are well separated and the sensitivity is probably adequate, particularly if a cathode ray polarograph is used. Some additional metals could also be determined—for example a method for the simultaneous determination of copper, lead and zinc has been developed by the Transvaal and Orange Free State Chamber of Mines Laboratory.

Mr. du Preez: The sample throughput of the atomic absorption method is so high that even allowing an hour for the instrument to warm up it is still possible to handle a hundred samples in a morning.

The polarographic method was not attempted for the determination of copper and zinc. I had the impression, however, that this method is slow and time consuming.

Dr. Matic: With a cathode ray polarograph the method can be very rapid.