

A
CRITICAL STUDY
OF
METHODS FOR THE DETERMINATION OF ZINC
IN
SOILS AND PLANT MATERIALS.

- by -

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INTRODUCTION.

Although it is only fairly recently that the importance of trace elements in plant nutrition has been established, enormous advances have been made in this field of research and numerous publications of considerable scientific and economic importance, dealing with the subject, have appeared.

The physiological functions of zinc in promoting plant growth have not all been satisfactorily identified as yet. There are indications, however, that zinc acts as a catalyst or regulator in plant metabolism. It appears too, that the element plays some role in chlorophyll formation. Zinc deficiency is associated with leaf chlorosis and a general collapse of vital processes.

The analysis of leaves and soils has become an essential part of the study of the nutritional diseases of plants and it is imperative that accurate and reliable methods of analysis should be available.

Such methods should have the following characteristics:-

They should be

- (a) accurate,
- (b) reproducible,
- (c) relatively short and
- (d) specially suited to routine analysis.

The analyst finds/.....

The analyst finds himself confronted with a large and ever-growing list of publications on the subject of the determination of zinc in soils and plants, indicating both the need for improvement and a general dissatisfaction with existing methods.

The published methods, though similar in many respects, contain a number of apparently conflicting recommendations, so that a thorough, critical examination of the published methods appears essential before any one of them can be applied to research into problems of nutritional deficiencies in plants.

This work was undertaken in order to investigate the accuracy and reproducibility of existing methods for determining zinc in soils and plant materials and where possible, to improve them. During the course of the research two methods were developed which conformed to the required standards of accuracy and reproducibility.

The methods for the determination of zinc in soils and plant materials fall into three main groups: colorimetric methods, the majority of which depend on the formation of the red zinc dithizonate complex, polarographic methods and spectrographic methods. As it was obviously impossible to test each of the published methods in the laboratory, the first task was to select, on the basis of available evidence, what appeared to be the best method in each group and to subject it to rigorous statistical tests.

The two/.....

The two most important factors governing the selection of an analytical method are accuracy and speed. These two factors are often, though not always, in conflict. When the accuracy of a method falls below a certain critical level, the results obtained thereby become of questionable value. This level of significant accuracy is not always easy to decide upon. In a field such as trace element nutrition, in which quantitative knowledge is still in the early stages of development, it appears unwise to set an upper limit on the desirable accuracy.

Other important considerations in the choice of an analytical method are the apparatus and funds available to the laboratory in which the work is to be carried out, as well as the time necessary to train laboratory assistants in the techniques of a particular method.

Bearing all these points in mind, though every effort was made to shorten methods, "short cuts" which endangered the ultimate accuracy of a method were regarded with suspicion.

For convenience a method was sought which would be equally applicable to the analysis of soils and plant solutions.

The work, as presented in this thesis, is divided into four main parts:-

Part I/.....

Part I deals with the critical study of colorimetric methods for the determination of zinc. As an outcome of this work, a modified colorimetric method was developed which proved to be both accurate and precise.

In Part II, studies on polarographic methods are presented. A new procedure was developed which proved to be shorter and simpler than previously published methods.

Part III contains the results of a statistical examination and comparison of the two methods.

In Part IV a survey is presented of the zinc content in the leaves of citrus and pineapples and in the soils on which they were produced. This survey, therefore, had two objects in view: to test the zinc method under routine laboratory conditions, on samples of widely differing soils and plants, and to learn something more about a nutritional deficiency which is widespread in the Union.

P A R T I.

THE COLORIMETRIC DETERMINATION OF ZINC IN SOILS
AND PLANTS.

1. REVIEW OF PUBLICATIONS ON THE COLORIMETRIC METHODS.

(a) THE USE OF DITHIZONE.

Most of the colorimetric methods for the determination of small concentrations of zinc depend on the reaction of this metal with di-phenylthiocarbazon, commonly known as dithizone, though recently the analogue of this compound, di-beta-naphthylthiocarbazon has also been recommended for this purpose.

(i) The Properties of Dithizone.

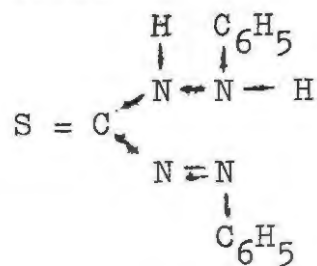
Sandell (1) records that dithizone was first prepared by Emil Fischer (2), who noted that the compound reacted with the heavy metals to give highly coloured products. However, the wide use of dithizone as an analytical reagent is due to Hellmut Fischer (3), who, in a series of papers on the properties of dithizone, laid the foundation of a great advance in the sphere of colorimetric analysis.

In an attempt to encourage the development in America of methods employing dithizone, H.J. Wichmann (4) published a review of the progress that had been made up to that time and suggested lines along which research could profitably be directed. He stressed particularly the need for a comprehensive study of the pH-dithizone-dithizonate equilibria.

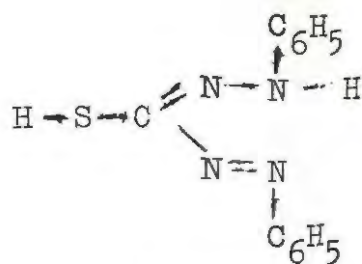
Dithizone exists/.....

Dithizone exists in both a keto and an enol

form:-

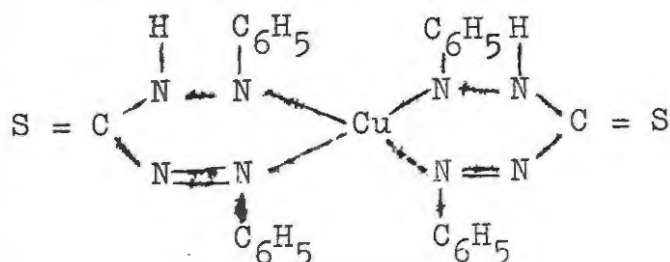


Keto.

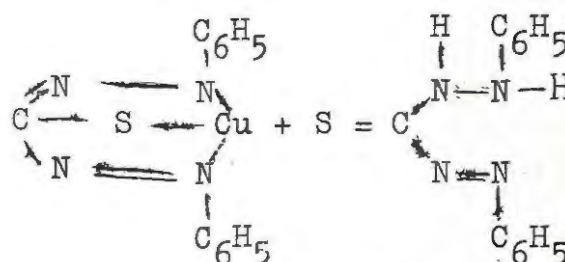


Enol.

Fischer (3) postulated that the keto forms of all the metal dithizonates existed, but that only copper, silver and mercury formed both keto and enol complexes, the keto tautomer being formed preferentially in acid or neutral solution and the enol form in alkaline solution.



Acid Solution.



Alkaline Solution.

Recently, however, Irving and Bell (5) have shown, by experiments involving the replacement of the H attached to the S by a methyl group, that only this hydrogen atom is replaced by metals when dithizonates are formed.

It is found that dithizone will react with the following metals to form coloured complexes: Mn, Fe, Co, Ni, Cu, Zn, Pd, Ag, Cd, In, Sn, Pt, Au, Hg, Tl, Pb, Bi. (Only the divalent forms of Fe, Sn, Pt react).

Thus it is/.....

Thus it is evident that the separation and determination, using dithizone, of zinc in complex solutions such as soil and plant extracts, presents a fairly complex problem. This problem has been tackled in two ways:-

- (1) Reagents such as cyanide, thiocyanate, thiosulphate and diethyl-dithiocarbamate are added to complex certain of the interfering elements.
- (2) The pH of the solution is adjusted so as to favour the extraction of only some of the elements concerned.

Both these separation techniques are normally included in dithizone methods, which consist essentially of a series of extractions, using dithizone dissolved in an organic solvent such as chloroform or carbon tetrachloride. The extractions are made at predetermined pH values from solutions containing the necessary complexing agents.

(ii) Solvents:

Both carbon tetrachloride and chloroform have been commonly used as solvent in dithizone extractions. In the work described here carbon tetrachloride was used exclusively. In addition to the fact that it is cheaper than chloroform, carbon tetrachloride has a number of advantages.

These are:-

- (a) Being denser than chloroform, its use gives rise to better and more speedy phase separations.
- (b) It has less tendency to form emulsions.

(c)/.....

- (c) It is not as volatile as chloroform. Therefore less care need be exercised in shaking a closed separatory funnel and volumes do not change appreciably during analysis.
- (d) The higher boiling point and greater stability of carbon tetrachloride make its recovery more easy.

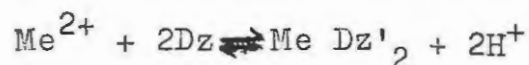
On the other hand dithizone and the dithizonates are not as readily soluble in carbon tetrachloride as in chloroform. This is particularly noticeable in the case of cadmium, but as this element is liable to interfere with the zinc determination, the lesser solubility of its dithizonate in carbon tetrachloride is likely to prove advantageous when this solvent is used.

Toxicity of Carbon Tetrachloride (6).

It should be noted here that carbon tetrachloride is a highly toxic organic solvent and for this reason, unnecessary exposure of the worker to its vapour must be avoided.

(iii) The Influence of pH on the Extraction.

The reaction between a bivalent metal ion (Me^{2+}) and dithizone (Dz) can be represented by the equation:-



(where Dz' represents the dithizonate ion, i.e. Dz which has lost one H by reaction with the metal).

If it is/.....

If it is assumed that all of the metal complex is taken into the organic phase, it is possible to calculate the amount of metal that will be extracted at any pH value under any fixed set of conditions from the relationship proposed by Koltoff and Sandell (7).

$$\frac{[\text{Me Dz}'_2]_o}{[\text{Me}^{2+}]_w} = K \frac{[\text{Dz}]_o^2}{[\text{H}]_w^2}$$

(where subscript "o" indicates the concentration in the organic phase and subscript "w" indicates the concentration in the aqueous phase).

This simple treatment has been shown to be valid in the case of the Zn-Dz-chloroform system. As, however, it disregards the distribution coefficients of the various compounds between the two phases and the deviation of activity coefficients from unity, calculations made using this expression cannot be expected to be exactly correct. Moreover, since other factors, such as the solvents used and the nature and concentration of complexing ions present, are liable to affect the value of K, the relationship is, as yet, of little practical value, especially as K has only been determined for one or two metals under a very limited range of conditions.

Wichmann (4) published a set of curves showing the relationship between pH and extraction by dithizone for a number of metals. He claims no accuracy for most

of these/.....

of these curves, however and explains that their shape was obtained by analogy with lead, the extraction curve for which was well-known, while their positions were fixed on the basis of fragmentary evidence gleaned from the literature.

Allan Walkley (8) found that zinc dithizonate was extracted completely from ammonium citrate solutions between pH 8 and 10 and hardly at all between pH 4 and 6. Hibbard (9) reported that zinc was quantitatively extracted between pH 7 and 10 while Cholak and his co-workers (10) found zero extraction at pH 6 rising rapidly to 100% extraction at pH 8.3 and then falling off rapidly with further increase in pH. On the other hand, in the method of analysis recommended by Sandell (1), the zinc extraction is carried out at pH 5 to 5.5.

In view of all these apparently conflicting results, the author decided to carry out a complete investigation of the pH-extraction relationships under the conditions of the analysis.

(iv) The Stability of Dithizone.

Sandell (1) and Wichmann (4) reported that dithizone was oxidised under weakly oxidising conditions to diphenylthiocarbo-diazone, which gives a yellow solution in chloroform and in carbon tetrachloride. Such conditions are brought about by the presence of ferric

iron/.....

iron (especially in basic solutions of citrate or tartrate and in alkaline cyanide solutions), by copper (especially in the presence of alkaline cyanides) and by small concentrations of halogens, permanganate, nitrous acid, etc. The oxidation of dithizone is apparently aided by sunlight and heat and for this reason solutions of the reagent are usually kept in dark bottles in the refrigerator.

The commercial product usually contains a little of the oxidation product. This is removed by making use of the fact that unlike dithizone itself, it is insoluble in dilute aqueous ammonia solution. In the method of purification, which has been described by Cowling and Miller (11), the carbon tetrachloride solution of dithizone is shaken with a dilute ammonia solution, when most of the dithizone is transferred to the aqueous phase. The original carbon tetrachloride is then discarded and the dithizone dissolved in a fresh volume of carbon tetrachloride, after the aqueous layer has been acidified.

(v) Mono-Colour and Mixed-Colour Procedures.

Once the red zinc complex has been extracted its concentration may be determined by visual or photometric comparison with standards. This may be done with or without the removal of the excess of green dithizone. Thus, in the mixed-colour method, the

colour/.....

colour of the extract solutions varies from deep red through purple to green, whereas in the mono-colour method, each solution is merely a different shade of red.

The extraction of the excess dithizone may be accomplished with the aid of dilute ammonia or sodium sulphide solution, according to Fischer and Leopoldi (v. Wichmann (4)).

The mono-colour method is the more suitable for visual comparison. Where a spectrophotometer or a good photometer equipped with a green filter is available, however, this procedure has no advantage over the mixed-colour method, unless the extraction is carried out in the presence of oxidising impurities. (v. Heinen and Benne (12)). Sandell (1) recommended that, except in the presence of large concentrations of other heavy metals, the mixed-colour method be used and he drew attention to a number of disadvantages of the mono-colour method.

An additional disadvantage of the mono-colour method lies in the practical difficulty of repeatedly extracting a denser carbon tetrachloride solution with a lighter aqueous solution.

(vi)/.....

(vi) Critical Review of Published Colorimetric Methods in which Dithizone is Used.

Sandell (1) described both a mono-colour and a mixed-colour procedure, recommending the latter for the analysis of rocks, soils and biological materials, though he suggested that the mono-colour method might be more suitable when large concentrations of other dithizone-complex forming metals were present.

In the mixed-colour procedure the zinc is extracted with a carbon tetrachloride solution of dithizone from a sodium acetate solution, buffered at pH 4.75 and containing sodium thiosulphate to prevent interference from other ions.

In the mono-colour method (originally due to Fischer and Leopoldi (3)) much the same procedure is followed, except that potassium cyanide is added to complex cobalt and nickel and the extraction is carried out at pH 5 to 5.5. After washing the carbon tetrachloride extract with an aqueous sodium thio-sulphate-sodium acetate solution, the excess dithizone is removed by repeated extraction with an 0.04% sodium sulphide solution.

This method is recommended by Prince (13) for plant material solutions, but for soil extracts he recommends a preliminary dithizone extraction after the addition of ammonium citrate and adjustment of

the pH/.....

the pH to about 7. This is followed by the transfer of the zinc to the aqueous phase with 0.02N hydrochloric acid.

The more important criticisms of the methods described by Sandell are:-

- (1) The principal dithizone extractions are carried out at pH values that have been shown to be unfavourable to the quantitative removal of zinc.
- (2) Thiosulphate, as Sandell has pointed out, affects the extraction very markedly.
- (3) Any oxidising agent (such as Fe^{3+}) in the complex sample solution is likely to react with the dithizone giving rise to inconsistent results. (In the mono-colour method this effect would not be as pronounced, as the oxidation product of dithizone is light coloured).
- (4) In the mono-coloured method the washing procedure to remove excess dithizone is tedious.

Cowling and Miller (11) published a method in 1941 involving three separate extractions. The zinc is first extracted with a carbon tetrachloride solution of dithizone from a solution buffered at pH 8.5 to 9.0 with ammonium citrate. This extraction separates the zinc and other heavy metals from iron, calcium, aluminium, etc. Secondly the zinc is separated from the other metals which form dithizone complexes, principally copper, by extraction with a dilute hydrochloric acid solution. Finally the zinc is extracted once more with dithizone after bringing

the acid solution/.....

- (6) They recommended the use of amber glassware to prevent the decomposition of dithizone due to the effect of sunlight.

In 1949 Shirley, Benne and Miller (17) published a shortened method based on the work of Cowling and Miller. In this method the pH of the solution is first adjusted to between 2 and 2.3 and then extracted with dithizone to remove copper. The carbon tetrachloride layer is discarded. The solution is then made alkaline by the addition of a buffer solution, carbamate solution is added and the zinc extracted with dithizone reagent. Excess dithizone is finally removed by shaking with 0.01N ammonia solution after the removal of the aqueous layer with a special syphoning apparatus. (It is doubtful whether this procedure is more convenient than using a second separatory funnel).

The report published by Heinen and Benne (12) in 1951 introduced no fundamental change in the above method. The authors made certain observations on the method of preparation of the sample. They recommended the use of a stock solution of carbamate, as suggested by Shirley and his co-workers (16) in 1948, and a more dilute solution of dithizone.

The method published by Shirley, Benne and Miller certainly presented the most important challenge to the original Cowling and Miller method, the method which was finally selected for study for this thesis.

From a cursory/.....

From a cursory examination of their results, however, it would appear that the analysis is not very precise and there is greater likelihood of interference from cobalt and nickel. These elements, as Sandell (1) pointed out, are not extracted from acid solution by dithizone, but their dithizonates, which are readily formed in alkaline solution, are not easily decomposed by acid. Thus the method of Cowling and Miller provides a separation from these elements, while that of Shirley, Benne and Miller does not.

Because the final extraction is made from a complex sample solution, which has been modified only by the extraction of copper, a one-colour photometric procedure becomes necessary. The removal of the excess of dithizone, which was shown to be unnecessary in the method of Cowling and Miller, by both its authors and by Shirley et al. (16), brings in an extraction procedure quite as tedious and likely to introduce errors as the first extraction in the latter method.

An independent method has recently been published by E.V. Arinushkina and A.I. Makarova (17). In this method the sesqui-oxides are precipitated by the basic acetate method, the copper extracted with dithizone from dilute hydrochloric acid solution and the zinc extracted with dithizone after adjustment of the pH

to 4.5/.....

to 4.5 in the presence of sodium acetate. Finally the excess dithizone is removed by extraction with 1:200 ammonia solution.

One hardly need draw attention to the fact that precipitation of hydrated oxides in the presence of trace elements is likely to cause losses by adsorption. Moreover, this step alone is likely to make the method more tedious than any of the others. Otherwise the method is similar to that described by Shirley, Benne and Miller.

(b) THE USE OF etc.

(b) THE USE OF DI-BETA-NAPHTHYLTHIOCARBAZONE.

Wichmann (4) suggested that analogues of dithizone might prove to be useful in this field. Such an analogue is di-beta-naphthylthiocarbazone (DN).

Cholak and his co-authors (10) have found the compound to be suitable for the determination of zinc in biological materials. They investigated the transmission curves for both the pure compound and the zinc complex in chloroform and as a result, recommended that photometric readings be taken at 550 m μ (minimum in curve for zinc complex) or 650 m μ (minimum in curve for pure DN).

They found that DN gave a quantitative extraction over the pH range 8 to 10.5, compared with the narrow range of complete extraction which they attributed to dithizone. In addition, these workers showed that there was no appreciable interference with the zinc extraction due to carbamate.

The method recommended by these workers involves the extraction of zinc from the sample solution with a chloroform solution of DN, after the former has been buffered to pH 9.5 by the addition of ammonium citrate buffer containing carbamate.

The zinc is then separated from the organic phase by shaking with 50 ml. of 0.2N HCl and the chloroform solution is discarded. After washing down with pure chloroform, an aliquot of a second buffer solution is added to
bring the pH/.....

bring the pH back to 9.5 and the zinc is again extracted with DN. Its concentration is then determined photometrically. (An alternative method is also described in which the zinc is determined polarographically in the HCl solution after the second extraction step).

Watson (19) used the method of Cholak, with minor modifications, to determine zinc in apple leaves and found it satisfactory. He compared results obtained by colorimetry, using the Lovibond Tintometer, with polarographic analyses on the same samples.

Martin (20) studied the use of DN for the extraction of zinc and his investigations of the effect of pH on the complexing of both zinc and copper are very enlightening.

2. THE METHOD OF etc./.....

2. THE METHOD OF COWLING AND MILLER.

In 1941 Cowling and Miller published a photometric dithizone method for the determination of small amounts of zinc in plant materials. A description of the method follows:-

Glassware:

Pyrex glassware was used for all vessels in which solutions had to be stored for more than a few hours. All vessels were thoroughly cleaned before use,

Reagents:

Carbon Tetrachloride:

Technical carbon tetrachloride was dried with anhydrous calcium chloride and then distilled in the presence of a small quantity of calcium oxide. Used carbon tetrachloride was reclaimed by distillation in the presence of a small amount of sodium hydroxide and sodium thiosulphate. It was then redistilled after drying in the same way as fresh technical carbon tetrachloride.

Standard Zinc Solution:

A 1000 ppm zinc solution was prepared by dissolving 0.25 g. of pure zinc in dilute sulphuric acid and making the solution to 250 ml. This was diluted to give a 10 ppm standard solution.

Ammonium Hydroxide:

Concentrated ammonia solution was distilled into cooled zinc-free water and thereafter diluted to give a 1N solution.

Hydrochloric Acid:/.....

Hydrochloric Acid: ,

A constant boiling point mixture of hydrochloric acid and water was distilled and the distillate diluted to give a 1N solution.

Dithizone Reagent:

This was prepared by dissolving 0.2 g. of dithizone in a litre of carbon tetrachloride. This solution was then shaken with dilute ammonia solution and the carbon tetrachloride discarded after most of the dithizone had been transferred to the aqueous phase. The solution was then acidified and the dithizone redissolved in pure carbon tetrachloride the solution being finally diluted to give a .01% (w/v) solution.

0.5M. Ammonium Citrate Buffer:

226 grams of dibasic ammonium citrate was dissolved in 2 litres of water and concentrated ammonia added to bring the pH to 8.5. The solution was then extracted with excess dithizone to remove all traces of zinc.

Carbamate Reagent:

0.25 g. of sodiumdiethyl dithiocarbamate was dissolved in 100 ml of zinc-free water. This solution was prepared fresh daily.

Solution A.

1 litre of 0.5M Ammonium citrate plus 140 ml of N. Ammonia solution were diluted to 4 litres.

Solution B./.....

Solution B.

1 litre of 0.5M Ammonium citrate plus 300 ml of N. Ammonia solution were diluted to 4.5 litres.

Just before use one volume of carbamate solution was added to 9 volumes of this solution to give the required volume of solution B.

0.02N Hydrochloric Acid:

This was prepared by dilution from the 1N solution.

Procedure:

The HCl solution of the ash from 5 grams of plant material is filtered into a 100 ml volumetric flask and neutralised with ammonia solution. 4 ml N hydrochloric acid are then added and the solution made to the mark.

FIRST EXTRACTION:

Pipette an aliquot of solution containing not more than 30 µg. of zinc into a 125 ml separatory funnel. Add 1 ml of 0.2N hydrochloric acid for each 5 ml of plant ash solution less than 10 ml taken, or 1 ml of 0.2N ammonium hydroxide for each 5 ml over 10 ml taken. Add 40 ml of solution A and 10 ml of dithizone reagent. Shake vigorously for half a minute to extract from aqueous phase the zinc and other dithizone-complex-forming metals which may be present. Then allow the layers to separate. An excess of dithizone, indicated by a yellow or orange aqueous phase, must be present. Shake down the drop of carbon tetrachloride extract from the surface and draw off the carbon tetra-

chloride/.....

chloride extract into a second separatory funnel as completely as possible without allowing any of the aqueous layer through. Rinse down the carbon tetrachloride extract from the surface of the aqueous layer with a 1 - 2 ml portion of clear carbon tetrachloride. Then run this carbon tetrachloride into the second funnel. Repeat this rinsing process until the extract has been completely transferred to the second funnel. Add 5 ml of clear carbon tetrachloride to the first funnel and shake for half a minute. When the layers separate, the carbon tetrachloride phase will have a clear green colour if all the metals which form dithizone complexes have been completely extracted. If this is not the case, repeat the process until the last extract is clear green, each extract being flushed into the second funnel with pure carbon tetrachloride. Discard the aqueous layer.

SECOND EXTRACTION:

Pipette 50 ml of 0.02N hydrochloric acid into the separatory funnel. Shake vigorously for $1\frac{1}{2}$ minutes and allow the layers to separate. Shake down the drop from the surface and run off, as completely as possible, the carbon tetrachloride phase, which should contain all the copper, without losing any of the aqueous phase, which contains the zinc. Rinse down the carbon tetrachloride extract from the surface of the aqueous phase and rinse out all remaining traces of green carbon tetrachloride from the funnel and stopcock with small portions of the pure solvent/.....

solvent. Shake down the carbon tetrachloride from the surface and run out the carbon tetrachloride as completely as possible. Remove the stopper from the funnel and place it across the neck of the funnel until the remaining carbon tetrachloride on the surface has evaporated.

FINAL EXTRACTION:

Pipette 50 ml of solution B and 10 ml of Dz reagent into the 50 ml of 0.02N hydrochloric acid solution. Shake for 1 minute and then allow the phases to separate. Flush out the stopcock and stem of the funnel with about 1 ml of the carbon tetrachloride extract and run the remainder of the extract into a test tube. Pipette 5 ml into a 25 ml volumetric flask and dilute to the mark with pure carbon tetrachloride. Determine the percentage light transmission of the solution with a photo-electric colorimeter equipped with a green filter. Readings should be taken not later than 2 hours after the final extraction.

The authors found that a zinc dithizonate - carbon tetrachloride solution showed a maximum absorption at a wavelength of 535 μ .

They demonstrated too, that there was remarkably little interference from other elements. The precision of their results appears to be good, but they made no attempt to examine them statistically.

Though the/.....

Though the sodium diethyl dithiocarbamate caused the optical density of the final zinc dithizonate solution to be slightly lower than when this compound was not used, Cowling and Miller claimed that provided the conditions were carefully controlled for both the standards and the actual analyses, this did not affect the final results.

3. EXPERIMENTAL, etc.

3. EXPERIMENTAL STUDIES ON THE DITHIZONE METHOD.

(a) THE PREPARATION of STANDARD ZINC SOLUTIONS.

The first problem was the preparation of an accurate standard zinc solution.

Some workers in this field (e.g. Walkley (21)) have recommended that A.R. zinc sulphate be used, but most workers (e.g. (11), (14), (15)) have described the preparation of standard solutions by dissolving pure zinc in sulphuric acid.

The dependance of the composition of the hydrated sulphate on slight changes in atmospheric conditions, as phase diagrams show, would seem to preclude its use as a standard. An attempt was made however, to prepare a standard stock solution from "specpure" zinc sulphate. Checks on the zinc concentration, made using the gravimetric ammonium phosphate method and the volumetric ferro cyanide method, disagreed so widely with one another and with the concentration calculated from the weight of salt taken, that this method was finally discarded.

Next, an attempt was made to prepare a standard solution by dissolving pure zinc in sulphuric acid. This, however, proved most difficult. Various concentrations of acid and various forms of the metal (A.R. grade granulated, pellets and powder) were tried, but in no case could complete solution of the metal be
obtained/.....

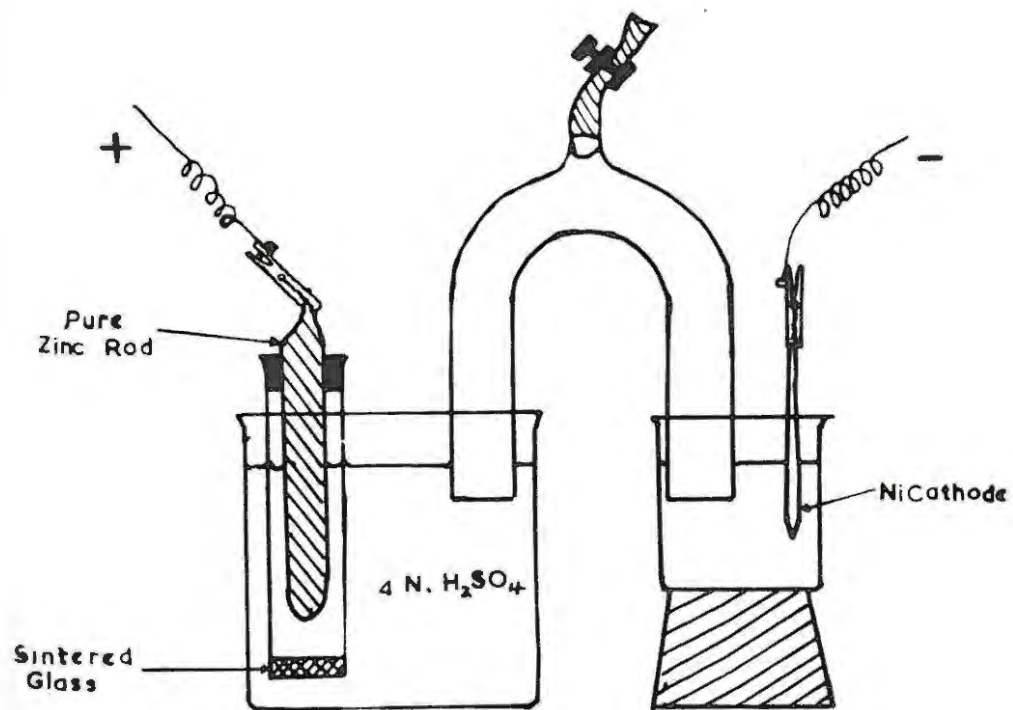


FIG. 1.

ELECTROLYTIC SOLUTION OF ZINC

obtained in less than four or five days, even when the mixture was heated to boiling on a hot plate. Reference to standard works on inorganic chemistry bore out the fact that pure zinc is not readily soluble in sulphuric acid. It must be concluded that the authors, who described the preparation of standard solutions by dissolving the metal, either omitted some detail or that the zinc they used was impure. (It was thought inadvisable to introduce oxidising agents to aid in the solution as these might have exerted an interfering influence on the colorimetric method by oxidising the dithizone).

To overcome this difficulty an electrolytic method was developed. The experimental arrangement for this method is shown diagrammatically in Figure 1.

The anode compartment, consisting of a 500 ml beaker containing 4N sulphuric acid, was connected by means of a U-tube bridge to the cathode compartment, a 150 ml beaker also containing 4N sulphuric acid. The anode, a pure zinc rod prepared by fusion of A.R. zinc granules, was enclosed in a glass tube, the lower end of which was closed with a sintered glass disc. This arrangement was found necessary to prevent too free a diffusion of the zinc ions through the cell. For the cathode a bright, pure nickel spatula was used.

Procedure./.....

Procedure.

The cathode and anode were cleaned, dried and accurately weighed. The apparatus was then set up as in Figure 1 and the cell was connected to a 30 volt D.C. supply. A current of 0.9 amps. was passed through the cell for three and a half hours. (It was found necessary to keep the current below one amp with the aid of a rheostat in the circuit as otherwise the circuit was broken by the boiling of the electrolyte in the U-tube).

After this the electrodes and the bridge were removed and carefully washed with distilled water. The electrodes were then dried and weighed again.

In order to determine whether an appreciable amount of zinc had diffused to the cathode compartment, the total volume of the contents of this compartment was roughly measured, and an aliquot analysed for zinc by the dithizone method, comparison being made with standards prepared from zinc oxide. The analysis showed that only about one tenthousandth of the weight of zinc that was dissolved migrated to the cathode compartment. This was considered to be a small enough quantity to be neglected.

The contents of the anode compartment were evaporated to a smaller volume and then made up to 500 ml in a measuring flask. Gravimetric analysis

by the ammonium/.....

by the ammonium phosphate method gave a result which agreed to within one part in a thousand with that obtained from the loss in weight of the anode (see Table of Results, I). A suitable portion of this stock solution was neutralised with redistilled ammonia and diluted to 2 litres to give a 100 ppm standard zinc solution.

TABLE OF RESULTS, I.

(a) Electrolytic solution of Zinc.

Weight of zinc anode before electrolysis. 115.5350 g.
Weight of zinc anode after electrolysis. 110.9478 g.
∴ Weight of zinc dissolved = 4.5872 g.

(b) Analysis of Zinc in Anode Compartment.

Volume of solution in anode compartment = 120 ml.
Aliquot taken for analysis = 20 ml.
Concentration of zinc found = 3.75 µg/ml.
∴ Total weight of zinc in anode compartment = $\frac{120 \times 3.75}{10^6}$ gms.
= 0.45×10^{-3} gms.

i.e. less than $\frac{1}{10,000}$ of the total zinc dissolved.

(c) Gravimetric Analysis of Zinc Stock Solution.

Volume of aliquots taken for analysis = 20 ml.
Weight of zinc found (a) 0.1834g. ∴ in 500ml. (a) 4.585 g.
(b) 0.1833g. " " (b) 4.583 g.
(c) 0.1834g. " " (c) 4.585 g.

Comparison with etc./.....

Comparison with Standard Solutions prepared from Zinc Oxide.

Although this method of preparation of a standard solution appeared to be reliable, it is not particularly suited to routine work and for this reason comparisons were made with solutions prepared by dissolving A.R. zinc oxide in acid. Solutions prepared in this manner were tested by the gravimetric method as well as by comparison of the diluted standards polarographically and photometrically (see Table of Results, II).

TABLE OF RESULTS, II.

Preparation of Solution.

(a) Weight ZnO taken = 0.2491 g.

Dissolved in acid and made to 1 litre.

∴ Zinc Concentration = .2001 grams/litre.

or 200.1 ppm.

(b) Gravimetric Check on Concentration of Zinc.

100 ml. aliquots taken for analysis.

	Wt. of Ppt., $Zn(NH_4)PO_4$	∴ Wt. Zn in 100ml.	∴ Wt. Zn/litre.
1.	0.0546 g.	0.02001 g.	.2001 g.
2.	0.0545 g.	0.01997 g.	.1997 g.
3.	0.0546 g.	0.02001 g.	.2001 g.

(c)/.....

(c) Comparison Between Solutions Prepared by the Two Methods.

A. COLORIMETRIC COMPARISON.

Nominal Wt. Zn.	Photometric Readings.	
	Solution 1.	Solution 2.
25 μ g.	.703	.700
25 μ g.	.699	.701
20 μ g.	.600	.598
20 μ g.	.600	.601

B. POLAROGRAPHIC COMPARISON.

Nominal Wt. Zn.	Wave Heights (mm.)	
	Solution 1.	Solution 2.
50 μ g.	24.5	24.7
50 μ g.	24.6	24.4
40 μ g.	19.0	19.1
40 μ g.	19.2	18.8

It was concluded that standard solutions prepared from A.R. zinc oxide were quite satisfactory. The following method is therefore recommended for preparing standard zinc solutions for trace-element analysis:-

Recommended Method for the Preparation of Standard Zinc Solutions.

Heat some A.R. zinc oxide powder to constant weight in a platinum crucible. Cool in a dessicator and weigh out 0.2488 g. Dissolve by warming with 10 ml. of N. sulphuric acid in water. Cool and make to 1000 ml. in a volumetric flask. This gives a 200 ppm stock zinc solution which should be stored in a polythene bottle.

A 5 ppm/.....

A 5 ppm. solution is prepared from this stock solution for use in preparing calibration curves for the analysis. The diluted standard should also be stored in a polythene bottle.

(b) STORAGE OF SOLUTIONS.

The majority of workers in this field have recommended the use of pyrex glassware wherever possible and particularly for all bottles in which solutions are stored. More recently, however, polythene bottles have become popular for the storage of solutions to be used in trace element work (22).

It was found that the ammoniacal buffer solutions required for the dithizone method, became contaminated after a few weeks storage in pyrex bottles. This was remedied at first by coating the inside of the bottles with a layer of pure paraffin wax, but subsequently polythene bottles have been used and found entirely satisfactory. All solutions used in the analysis were stored in polythene bottles except the N hydrochloric acid which was found to keep quite satisfactorily in a pyrex bottle.

Gatos (23) reported that storage of solutions in polythene ware resulted in a depression of waves and of maxima in polarographic analysis. No such interference was observed in this work.

(c)/.....

(c) TRANSMISSION CURVES OF CARBON TETRACHLORIDE SOLUTIONS OF DITHIZONE AND ZINC DITHIZONATE.

It was considered necessary to plot the transmission curves for solutions of dithizone and zinc dithizonate in carbon tetrachloride, before selecting a suitable light filter for use with the Spekker photometer. Although Cowling and Miller published the transmission curve for zinc dithizonate, they made no mention of the spectral transmission properties of dithizone solutions. It is extremely important when dealing with a mixed-colour method to know the transmission properties of both the coloured solutions.

Shirley et al. (16) published transmission curves for dithizone, zinc dithizonate and a mixture of the two in carbon tetrachloride. The difference in the conclusions reached during the work reported here amplifies the importance of such an investigation.

(i) Zinc Dithizonate.

The solution was prepared by repeatedly shaking 10 ml. of a 0.01% dithizone solution with excess zinc in ammonium citrate solution at pH 8.5. To ensure that there was no excess dithizone, the carbon tetrachloride solution was finally extracted with zinc-free 0.01N ammonia solution. The carbon tetrachloride solution was then run into a 50 ml. volumetric flask, which was then filled to the mark with the pure solvent.

(ii)/.....

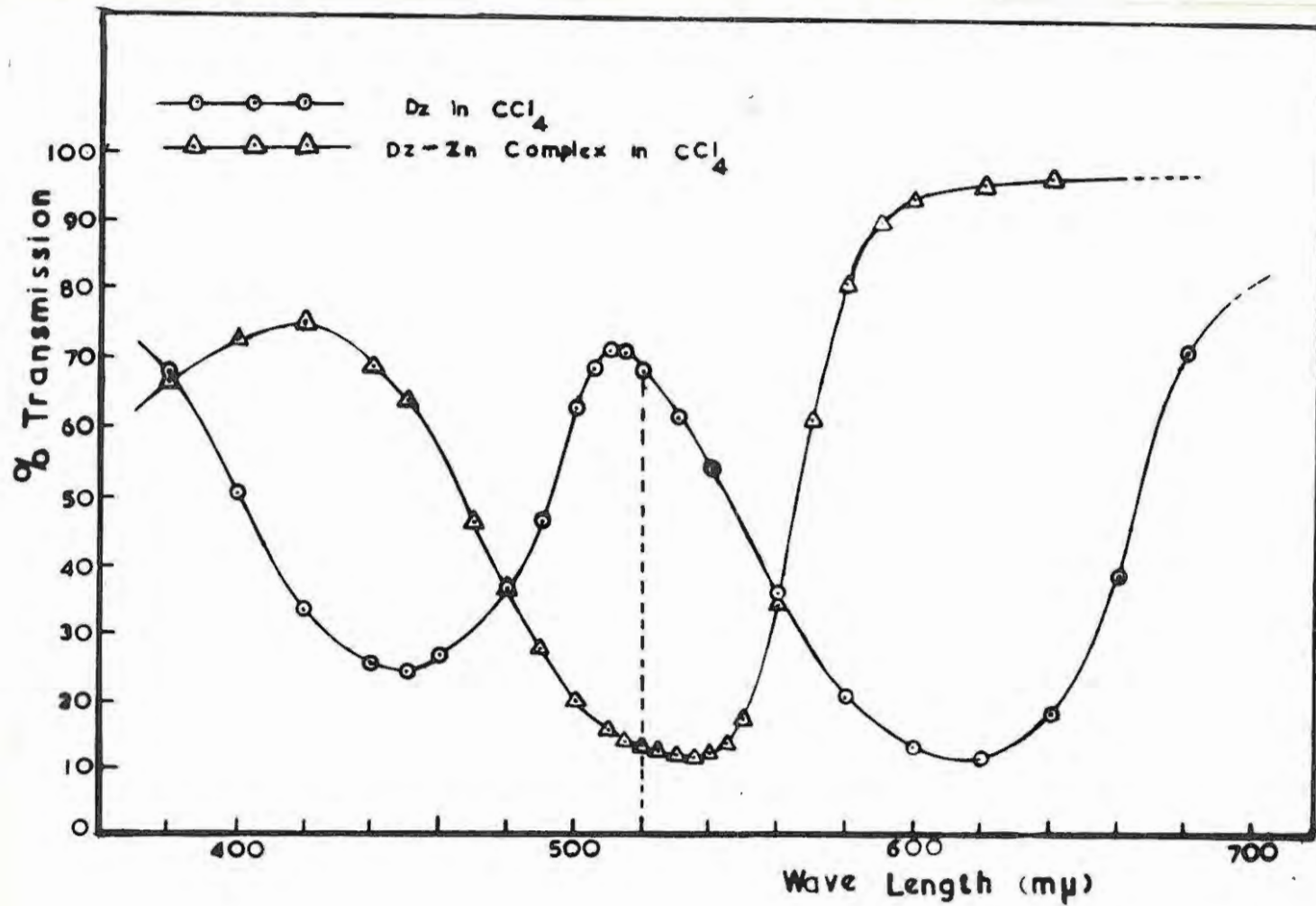


FIG. 2.
TRANSMISSION CURVES OF DITHIZONE & ZINC DITHIZONATE

(ii) Dithizone.

10 ml. of 0.01% dithizone solution were pipetted into a 50 ml. volumetric flask and made to the mark with carbon tetrachloride.

Transmission measurements were made using the Beckman Model D.U. Spectrophotometer and the results recorded in the form of a graph. (Figure 2).

Selection of the most suitable Wave Length for Transmission Measurements.

It is normal in colorimetric analysis, to make the transmission measurements at a wave length corresponding to a minimum in the transmission curve of the metal-complex-solvent system. Following this general principle, previous workers (e.g. Cowling and Miller (11)) have recommended that measurements be made at 535 μ . In mixed-colour methods, however, it is more often recommended that measurements be made at that wave length where the separation between the transmission curves for complexing agent and metal complex is greatest.

In the method of/.....

In the method of Cowling and Miller not all of the excess of dithizone remains in the carbon tetrachloride solution, as a proportion of it is transferred to the ammoniacal aqueous layer. The excess is, moreover, affected by factors such as deterioration of the reagent solution and oxidation during the extraction. Thus the method may not be regarded as a true mixed-colour method, in which the concentration of excess reagent is inversely proportional to the metal being determined.

It is important therefore, to select a wave length at which the light absorption due to the unchanged dithizone is small.

To assist in the selection of the most suitable wave length, the following table (Table III) was drawn up.

TABLE III.
QUANTITATIVE EXAMINATION OF TRANSMISSION CURVES.

Wave length (μ)	Transmission of Dz. T'	Transmission of Zn Complex T	Separation = T' - T	Loss in Sensitivity T - T _{min.} †
505	68.5%	17.5%	51.0%	5.7%
510	71.0	15.9	55.1	4.1
515	70.8	14.2	56.6	2.4
520	68.2	13.3	54.9	1.5
525	65.0	12.6	52.4	0.8
530	61.9	12.0	49.9	0.2
535	57.5	11.8	45.7	0.0

† T_{min} = Transmission of carbon tetrachloride solution of zinc dithizonate at 535 μ .

Examination of/.....

Examination of the table indicates that either 515 or 520 m μ would be the most suitable. 520 m μ was eventually selected. At this wave length maximum separation is nearly attained, while the reduction in sensitivity^{due} to the zinc dithizonate colour is very small (1.5%).

A statistical examination of the results of 31 analyses, in which measurements were made using the Cenco Sheard Spectrophotometer both at 520 m μ and 535 m μ showed a standard deviation of 1.9% at the former and 2.1% at the latter wave length giving support to the choice of 520 m μ rather than 535 m μ .

(a) THE INFLUENCE OF HYDROGEN ION CONCENTRATION ON EXTRACTION EFFICIENCY.

(i) Dithizone Extraction of Zinc.

The advantages which Cholak and his co-workers (10) have claimed for di-beta-naphthylthiocarbazono as a reagent for zinc, depend largely on the wider range of pH permissible during the extraction with this reagent. Using a 150% excess of dithizone, these authors showed that zero extraction of zinc occurred at pH 6, slight extraction at pH 7 and then a rapid increase in extraction to 100% at pH 8.3. The extraction efficiency was shown to decrease rapidly again with further increase in pH.

Their results for dithizone disagree widely with observations made by Walkley (8) and by Hibbard (9)

in carrying out/.....

in carrying out similar investigations. Cholak and his co-authors suggested that his discrepancy might be due to the fact that they made their extractions from more complex solutions, citing the work of Biefield and Patrick (24) on the influence, on the dithizone extraction, of the presence of various anions. This, however, does not seem to be a valid reason, for an examination of Biefield and Patrick's work shows that the effect which they observed does not resemble that reported by Cholak et al. Their results show little change in the shape of the pH extraction curves for lead, but slight pH shifts. These workers have, moreover, used the ions, cyanide, acetate, tartrate and citrate in their investigations, ions whose strong complexing properties are well-known.

In the paper of Cholak et al. full details are not given of how the points on their dithizone extraction curve were arrived at. If however, as is indicated in the paper, the zinc in the chloroform phase was determined colorimetrically by the mixed-colour method, an explanation for their results can readily be found, because the proportion of excess dithizone which dissolves in the aqueous phase increases rapidly as the pH rises above 8.

Martin (20)/.....

Martin (20) suggested that the disagreement between the results of Cholak and his co-workers and those obtained by other workers may be due to the abnormally high citrate concentration which the former used.

When investigating a method which involves two extractions with dithizone, like the methods of Cowling and Miller and of Cholak, there seems little justification for investigating the effect of pH on the extraction of zinc from a complex solution. In the first step repeated extractions with reagent are made, which, even on the basis of Cholak's curve, should produce complete extraction over a wide pH range. In the second extraction, in which it is important to control the volume of reagent used, the zinc is extracted from a relatively simple solution containing ammonium, chloride, and citrate ions together with traces of those interfering elements which have not been completely removed.

It was considered, therefore, that an investigation of the pH extraction relationship should be made under the conditions existing in the second dithizone extraction.

There was the possibility too, that the use of carbon tetrachloride in place of chloroform, which had been used in previous investigations of the pH-extraction relationship, might introduce differences.

The procedure/.....

The procedure adopted for investigating the pH-extraction relationships was as follows:-

A previously determined quantity of N. ammonium hydroxide solution was added from a burette to 8 ml of 0.5M citric acid, contained in a 100 ml separatory funnel. The contents of the funnel were diluted to approximately 40 ml by the addition of the required volume of redistilled water from a measuring cylinder. The solution was then shaken for two minutes with 10 ml of dithizone reagent to remove any heavy metal impurities. The carbon tetrachloride layer was run out, rinsed down with pure carbon tetrachloride and discarded. 5 ml of a 5 ppm. standard zinc solution were then added and the extraction with 10 ml of dithizone reagent was repeated. This time the carbon tetrachloride layer was run into a second separatory funnel. The pH of the aqueous phase was measured by means of a Beckmann pH meter. Excess dithizone was removed from the carbon tetrachloride extract by repeated washings with 5 ml portions of a .04% aqueous sodium sulphide solution until the washings were colourless. The carbon tetrachloride layer was then run into a 50 ml volumetric flask, made to the mark with the pure solvent and the

optical density/.....

optical density of the resulting solution measured with a Beckmann D.U. Spectrophotometer.

In plotting curve (Figure 3), for purposes of comparison it was assumed that Beer's Law applied and that the highest optical density corresponded to 100% extraction. On the basis of this assumption, the results were found to agree fairly well with those of Walkley (8). Due to the method used for the purification of the dithizone, however, the excess of dithizone used for the extraction is uncertain. From the curve (Figure 3) it is clear that the extraction is very nearly quantitative between pH 7 and 10, but the maximum appears to be reached between pH 9 and 9.5.

(ii) Dithizone Extraction of Copper.

The pH-extraction relationship for copper dithizonate was also investigated. In this case, since a mono-colour method is not recommended, blank determinations were carried out at the same pH values and the differences in optical density between the extracts of the blanks and the solutions to which copper (25 μ g) had been added were plotted as percentages of the maximum (see Figure 3).

The method/.....

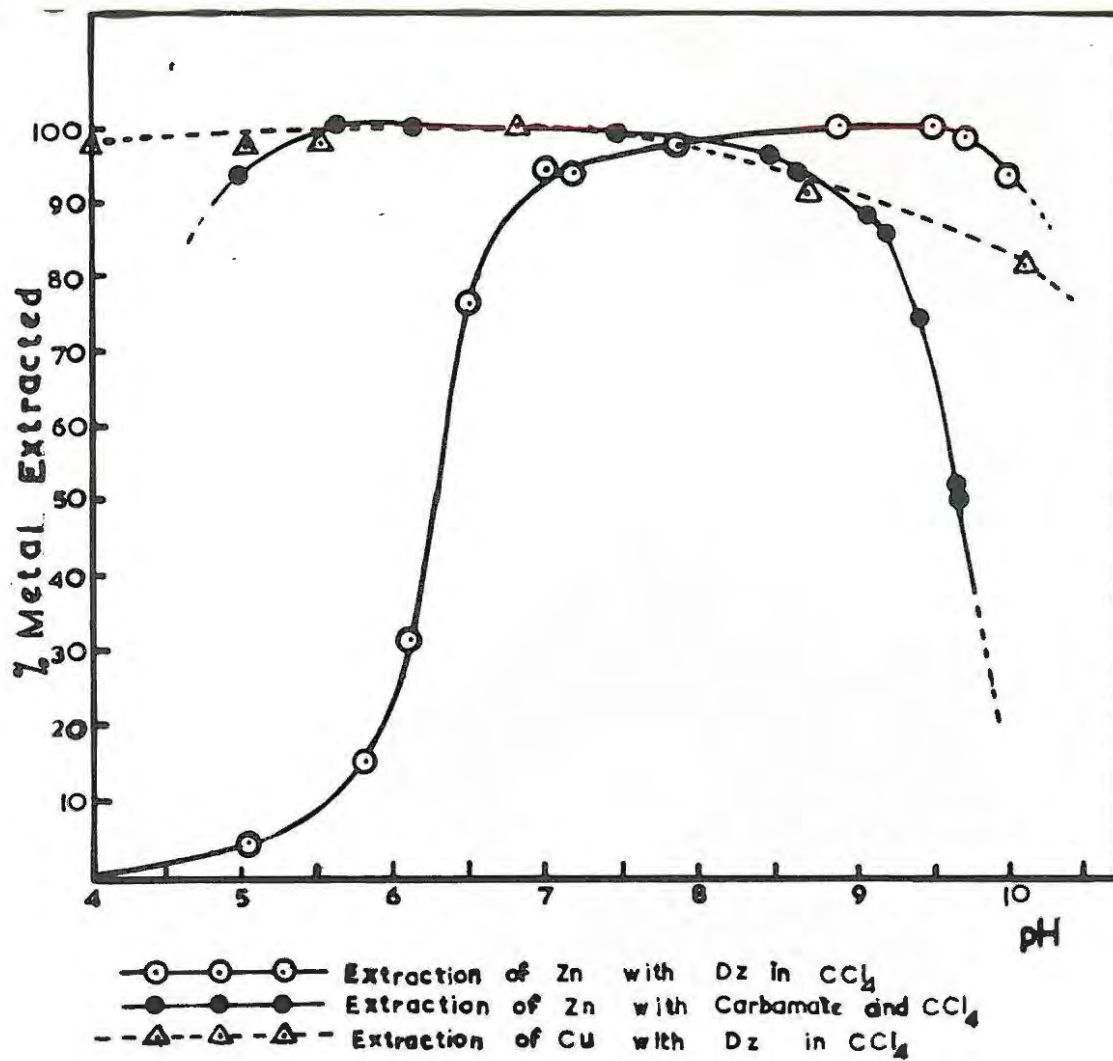


FIG. 3

pH-EXTRACTION RELATIONSHIPS

The method did not prove entirely satisfactory, but the results indicated an almost constant quantitative extraction of copper between pH 2 and 8, the extraction efficiency falling off somewhat above the latter pH.

(iii) Sodium diethyl dithiocarbamate Extraction of Zinc.

Martin (20) investigated the influence of pH on the complexing, by sodium diethyl dithiocarbamate, of both copper and zinc. His results showed a quantitative extraction of zinc (using chloroform as extractant) between pH 6 and 9 the extraction falling off rapidly at pHs below 6 and above 9. For copper he obtained a constant extraction of over 90% between pH 6 and pH 10.

As sodium diethyl dithiocarbamate is present during the final extraction in the method of analysis recommended by Cowling and Miller, the relationship between pH and the complexing efficiency of that compound has an important bearing on the selection of the optimum pH for extraction. For this reason an investigation was made of the extraction of zinc as the diethyl dithiocarbamate complex, in the absence of dithizone, but otherwise under the conditions of the final extraction step in the Cowling and Miller method.

The procedure/.....

The procedure adopted was similar to that followed in the investigation of the dithizone extraction. To the solution in the separatory funnel, containing 25 μg . of zinc, together with citric acid and sufficient ammonia to give the desired pH, was added 5 ml of a 0.2% solution of sodium diethyl dithiocarbamate. The extraction was then carried out by shaking for two minutes with 10 ml of pure carbon tetrachloride. The carbon tetrachloride layer was then run into a beaker and evaporated on a sand bath. The organic complex was destroyed by evaporation with 5 ml of nitric acid containing a few drops of perchloric acid. The residue was taken up in dilute acid and analysed for zinc by the dithizone colorimetric method.

The results of this investigation are also shown in Figure 3.

It was finally decided to carry out the dithizone extractions of zinc at a pH between 9.0 and 9.5 rather than at a pH of 8.5 - 9.0 as suggested by Cowling and Miller. This decision was made for the following reasons:-

- (a) The most efficient extraction of zinc by dithizone in carbon tetrachloride appears to take place between pH 9 and pH 9.5.
- (b) It has been shown that the extraction of zinc as the diethyl dithiocarbamate complex falls off rapidly at pH values above 9, while Martin (20) has shown that copper is strongly complexed up to pH 10.

(c)/.....

- (c) The extraction of copper dithizonate falls off somewhat as pH increases above 8.
- (d) Walkley (8) reported that the adsorption of zinc by suspended silica was appreciable at pH 8 but negligible at pH 9.5 to 10.

(e) STUDIES ON THE TECHNIQUE.

The next step was to develop the dithizone colorimetric method along practical lines using the published method of Cowling and Miller as starting point.

(i) Pipetting of Carbon Tetrachloride Solutions.

Carbon tetrachloride is an highly toxic organic solvent and for this reason pipetting by mouth is to be avoided. Pipette filling devices were found to be unsatisfactory due to the fact that carbon tetrachloride is a volatile and very dense liquid and therefore difficult to pipette accurately. Moreover carbon tetrachloride vapour is liable to attack any rubber connection to the pipette. As however, it is important to measure out accurately the volume of dithizone reagent used in the final extraction, an automatic dispensing pipette was modified for the purpose. The pipette which is shown in Figure 4 has a reservoir attached which holds sufficient to fill the pipette six times. The pipette is filled

by turning/.....

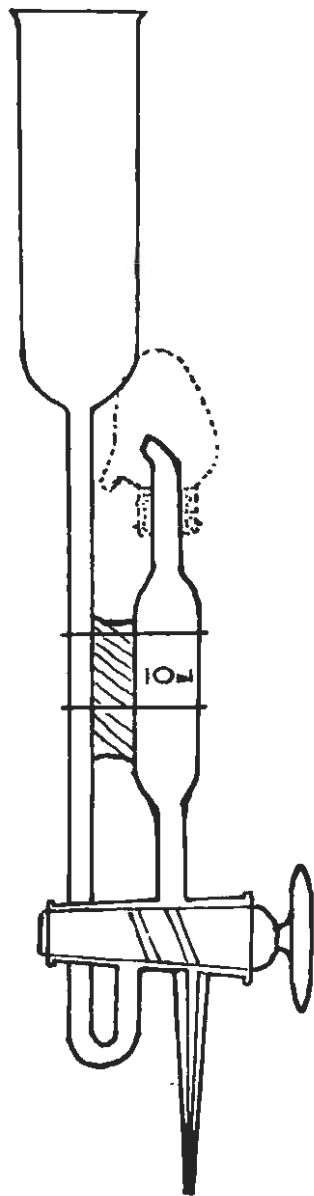


FIG. 4.

AUTOMATIC PIPETTE

by turning the tap to allow solution from the reservoir to run into the pipette until a drop runs out at the top. The glass bulb, supplied with the pipette in its original form, was retained to catch this overflow which, however, could readily be kept so small that it was only necessary to empty the bulb after pipetting six times. The stopcock was kept well ground in, so that it was unnecessary to use any grease. This pipette was found to be excellent for the rapid additions of precise volumes of dithizone reagent.

(ii) Preparation of Dithizonate Solution for Photo-
metry.

In the Cowling and Miller method 5 ml of the final dithizone-carbon tetrachloride extract are pipetted into a 25 ml volumetric flask and diluted to volume with pure carbon tetrachloride.

This procedure has a number of disadvantages:-

- (a) The difficulties of pipetting carbon tetrachloride as mentioned above.
- (b) It introduces a time consuming step into the analysis. The pipette must be rinsed with part of the solution and the operation must be carried out before any appreciable volume of the carbon tetrachloride has evaporated.
- (c) It is essential that no carbon tetrachloride from the previous extraction be left in the funnel. This involves carrying out a perfect phase separation, which is extremely difficult, as well as allowing time for the drop of carbon tetrachloride on the surface to evaporate.

In order to/.....

In order to avoid these difficulties, it was decided to transfer the complete carbon tetrachloride phase to a 50 ml volumetric flask at the end of the final extraction and then to dilute to volume. This was accomplished by running most of the carbon tetrachloride phase into the volumetric flask and then washing down with three 2 ml portions of pure carbon tetrachloride. In this way all the coloured carbon tetrachloride, including the drop on the surface of the aqueous layer, was readily transferred. The additional excess of dithizone from the aqueous phase, introduced in this way, was very small indeed.

This technique was found to be both time saving and to give more precise results than that proposed by Cowling and Miller.

(iii) Time of Extractions.

In the method described by Cowling and Miller, the first extraction of zinc and other heavy metals from the sample solution is carried out by shaking for half a minute with 10 ml of dithizone, followed by repeated half minute extractions with 5 ml portions of carbon tetrachloride until the organic phase has a pure green colour. The author found, however, that if, after the addition of the dithizone, the separatory funnel was shaken for two minutes, complete extraction of up to 25 μ g. of zinc was obtained and

the first/.....

the first extraction with pure carbon tetrachloride gave a pure green solution. On the other hand, when the first shaking time was only half a minute, several additional carbon tetrachloride extractions were necessary. Thus it was clear that equilibrium was not reached after half a minute of shaking. By increasing the initial shaking time to two minutes the total time for the analysis was actually reduced and the total volume of carbon tetrachloride used was smaller.

It is even more important that equilibrium between the phases should be attained in the final extraction and for this reason the shaking time for the final extraction was increased to two minutes.

For the sake of uniformity, the shaking time in the second extraction was also increased to two minutes. It is essential that as much as possible of the zinc be transferred from the dithizone-organic phase to the acid aqueous phase in the initial shaking period, as the carbon tetrachloride phase is discarded immediately afterwards.

(f) STABILITY etc./.....

(f) STABILITY OF DITHIZONE AND DITHIZONATE SOLUTIONS.

The colour of chloroform and carbon tetrachloride solutions of dithizone and the dithizonates have been observed to fade when exposed to sunlight. For this reason Shirley and his co-authors (16) recommended that amber glass separatory funnels and volumetric flasks be used during the analysis. Because this introduces a number of difficulties besides that of obtaining such glassware, it was decided to test the stability of the solutions under ordinary laboratory conditions in the following manner.

Three dithizone-dithizonate solutions were set aside in 50 ml volumetric flasks on the completion of a set of analyses. One was placed in a cupboard in the dark, one in indirect sunlight away from the window and the third in direct sunlight on the window sill.

The solution placed in direct sunlight faded to a pale yellow in two hours, but neither of the other two solutions, after a period of forty eight hours, showed any change in optical density that could be detected with a Beckmann model D.U. Spectrophotometer.

In view of these observations, no special precautions were taken to exclude sunlight, though all photometric readings were taken as soon as possible and,

where this/.....

where this step was unavoidably delayed, the solutions were kept in the dark.

The dithizone reagent solution was prepared about two weeks before it was required and kept in a brown bottle in the refrigerator. When these precautions were taken, it was found that the standard determinations need only be repeated every three days for very precise work.

4. THE MODIFIED etc./.....



4. THE MODIFIED DITHIZONE-COLORIMETRIC METHOD.

The Recommended Method for the Determination of Zinc in Soil and Plant Material Solutions.

Reagents.

(i) Water:

All the water used for preparing solutions and in the analysis should be redistilled from an all-pyrex-glass still. (All the "zinc-free" water used in the work for this thesis was obtained from the recycling still as developed by Faure, Faure and Gledhill (25)).

(ii) Carbon Tetrachloride:

Dry a suitable volume of "Technical Grade" carbon tetrachloride by shaking with calcium chloride in a 5 litre flask. Leave to stand for several hours, pour off and distil. The first and last fractions are kept separate from the main distillate for later redistillation. It is advisable to prepare seven or eight litres of the pure solvent in this way before undertaking a series of analyses.

Used carbon tetrachloride may be reclaimed by distillation after the addition of a small volume of dilute sodium hydroxide solution containing a little sodium thiosulphate. This distillate is washed with distilled water, dried and redistilled.

(iii)/.....

(iii) Ammonium Hydroxide:

Distil commercial "C.P." ammonia in an all-pyrex-glass still and collect the distillate in a polythene bottle cooled in ice water. Dilute a suitable volume of the distillate to give a 1N solution.

(iv) Hydrochloric Acid:

Distil a 1:1 (V:V) mixture of C.P. HCl and distilled water in an all-pyrex-glass still. Discard the first fraction and collect the distillate in a pyrex-glass flask once the temperature has remained steady for some minutes. This gives a constant boiling point mixture which is approximately 20% HCl. Dilute to give a 1N solution.

(v) Dithizone Reagent:

Dissolve 0.1 gram of diphenylthiocarbazone in 500 ml of carbon tetrachloride by shaking in a separatory funnel. Next add 20 ml of ^NNH₄OH in 1 litre of water to the funnel and, by vigorous shaking, transfer the dithizone to the aqueous phase. Discard the carbon tetrachloride layer and rinse the aqueous phase several times with the pure solvent. Add 500 ml of pure carbon tetrachloride, acidify by adding 50 ml of 1N zinc-free hydrochloric acid and redissolve the dithizone in carbon tetrachloride by shaking vigorously.

Dilute the/.....

Dilute the resulting carbon tetrachloride solution to 1 litre and store it in a dark glass bottle in the refrigerator.

(This solution is best prepared a week or two before required).

(vi) 10% Ammonium Citrate:

Dissolve 10g. of A.R. ammonium citrate in 100 ml zinc-free water.

(vii) Ammonium Citrate Buffer 0.5M.

Dissolve 226 grams of dibasic ammonium citrate in 2 litres of water and adjust the pH to 9 by the addition of concentrated ammonia solution (pH meter). Extract the solution with excess dithizone reagent and wash with carbon tetrachloride until free of heavy metals (i.e. until the carbon tetrachloride washings are green).

(viii) Carbamate Reagent:

Dissolve 0.2 grams of sodium-diethyl dithiocarbamate in 100 ml of zinc-free water.

This must be prepared fresh daily.

(Shirley et al. (16) have reported that this solution may be kept satisfactorily in the refrigerator, but, as the only refrigerator available to the author during the work for this thesis was set well below freezing point, it was out of the question to keep an aqueous solution in it).

(ix)/.....

(ix) Solution A.

Dilute 1 litre of 0.5M ammonium citrate buffer plus 150 ml of N ammonia to 4 litres.

(x) Solution B:

Dilute 1 litre of 0.5M ammonium citrate buffer plus 320 ml of N ammonia solution to 4.5 litres. Just before use, add 1 volume of fresh carbamate solution to 9 volumes of the above to obtain the required composition.

(xi) 0.02N Hydrochloric Acid:

This is prepared by dilution of the 1N solution.

NOTE: 4 litres of A, B and 0.02N hydrochloric acid together with 2 litres of dithizone reagent are sufficient for 80-90 analyses, allowing for a small wastage.

(xii) Standard Zinc Solution:

This is prepared from zinc oxide in the manner described on page 33.

Glassware:

The importance of cleanliness of glassware cannot be over emphasised. Separatory funnels may be cleaned at first with "chromic acid". After this, and between analyses, they must be rinsed repeatedly with zinc-free water. Stopcocks should be greased with a minimum of the purest grease available and only when absolutely necessary.

All other/.....

All other glassware should be kept as clean as possible and rinsed directly before use.

Hydrochloric acid solutions appear to keep well in pyrex glass, but all other solutions should be kept in polythene ware. Ammoniacal solutions, in particular, should not be kept in contact with glass for longer than is absolutely essential. Rubber contains an appreciable amount of zinc and its use must be avoided throughout.

Procedure:

It was found convenient to carry out six determinations simultaneously. Six sets of two separatory funnels were set up as shown in Figure 5.

Pipette an aliquot of the soil or plant solution, containing between 5 and 25 μg . of zinc into a 100 ml. separatory funnel. Add 1 ml. of 10% ammonium citrate solution, then one drop of 0.1% phenolphthalein solution and titrate with concentrated redistilled ammonia until just pink.

First Extraction:

(Separation of zinc and other dithizone-complex-forming metals from Fe, Al, Ca, etc.)

Add about forty ml. of solution A to the separatory funnel and 10 ml. of dithizone reagent (from the automatic pipette).

Shake vigorously for two minutes and allow the carbon tetrachloride layer to separate. (The aqueous layer should be yellow-orange due to excess Dz). Run the/.....

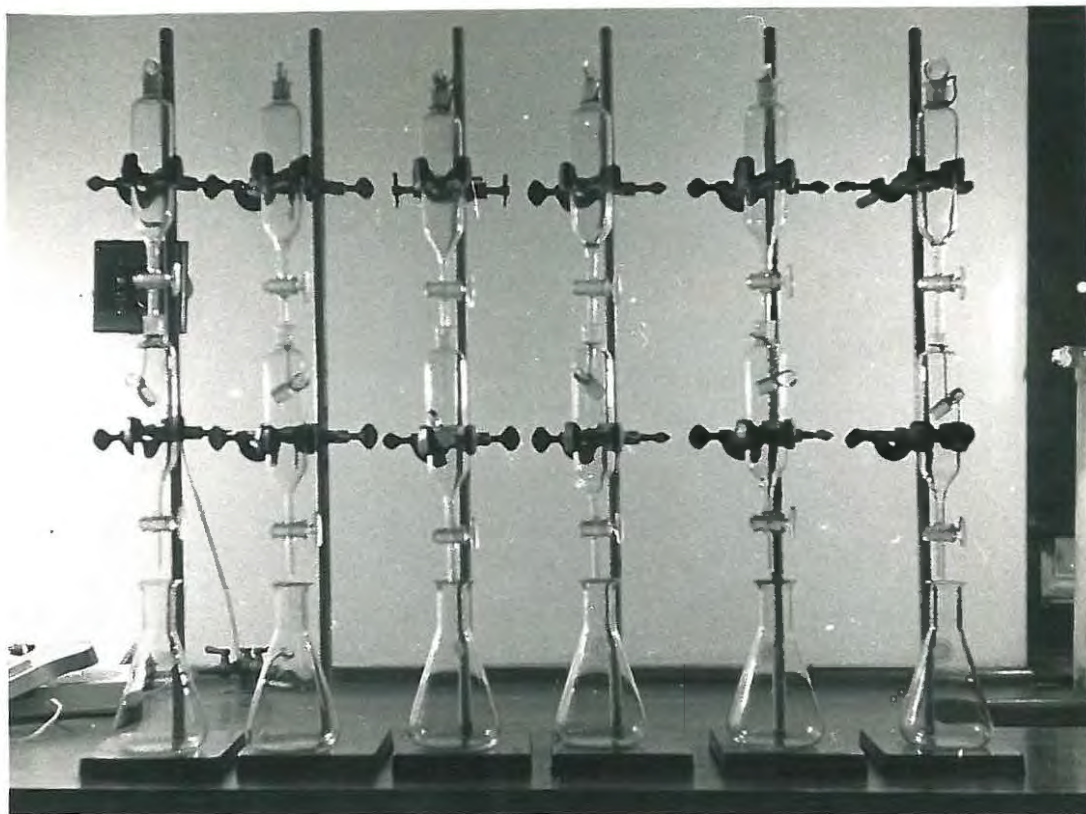


FIG. 5.

ARRANGEMENT OF SEPARATORY FUNNELS
FOR ROUTINE COLORIMETRIC ANALYSIS.

Run the carbon tetrachloride layer into a second separatory funnel, washing down with two or three 2 ml. portions of carbon tetrachloride. Then add 5 ml. of carbon tetrachloride and shake for $\frac{1}{2}$ minute. The carbon tetrachloride layer should now be pure green. (If not, repeat). The carbon tetrachloride is run into the second funnel and the first funnel is flushed with a small volume of the pure solvent as before.

Second Extraction:

(To separate zinc from the other dithizone-complex-forming metals).

Pipette 40 ml of 0.02N hydrochloric acid into the second funnel.

Shake vigorously for two minutes. Allow the phases to separate and then run out the carbon tetrachloride layer and discard it. The drop on the surface and other remaining carbon tetrachloride are flushed down with small portions of the pure solvent as before.

Next add 5 ml of carbon tetrachloride and shake for about fifteen seconds, allow the layers to separate and run out and discard the organic phase. (This step removes any carbon tetrachloride solution which might adhere to the sides of the funnel).

Third Extraction:

(To recomplex the zinc for colorimetric measurement).

Pipette into the separatory funnel 40 ml of solution B and 10 ml of dithizone reagent. Shake vigorously/.....

Shake vigorously for two minutes. Run the organic layer into a 50 ml measuring flask. Flush with three 2 ml. portions of carbon tetrachloride which should remove all the coloured carbon tetrachloride.

Make up to the mark with pure carbon tetrachloride.

Measure the optical density of the solution as soon as possible with a spectrophotometer at 520 m μ . If an ordinary photometer is used, insert a green filter.

Standard Curve:

To prepare a standard curve the procedure is carried out as above, 1 to 5 ml. of a 5 ppm zinc solution being run into the first separatory funnel instead of the aliquot of extract solution.

Discussion.

The main modifications introduced as compared with the original method of Cowling and Miller are as follows:-

- (i) The pH at which the zinc is extracted with dithi-zone is raised from 8.5 to 9-9.5. This change reduces the chance of interference from copper, the diethyl dithiocarbamate added, and suspended silica.
- (ii) The wave length at which optical density measurements are made is changed from 535 m μ to 520 m μ , a higher precision being obtained thereby.
- (iii) Many minor changes in the general technique were introduced, which make the method more precise, and more suitable for routine work.

5. STATISTICAL STUDY OF THE PRECISION OF THE MODIFIED DITHIZONE METHOD.

The next step was to test the precision of the modified method and to develop the technique for carrying out a large number of analyses. To do this, fifty analyses were carried out on aliquots of a soil extract solution prepared in the following manner.

100 grams of soil were ground as finely as possible in an agate mortar. This sample was divided into three separate portions, each of which was accurately weighed into a kjeldahl flask. 100 ml. of 2N sulphuric acid was then added to each and the flasks were gently heated over small flames for three hours, the volume being kept approximately constant during this period by the addition of water. The acid extracts were filtered hot and, after evaporation to a small volume, the united extract was finally evaporated to dryness in a platinum dish. Silica was removed by repeated additions of 5 ml. of hydrofluoric acid and evaporation on a sand bath.

Finally, the contents of the dish were dissolved in dilute nitric acid. The solution was diluted, neutralised, made to a litre in a volumetric flask and transferred to a polythene bottle.

5 ml. aliquots of this solution were used in the analysis. Photometric measurements for each analysis were made with a Cenco Sheard "Spectrophotometer" at the

wave lengths/.....

wave lengths 520 m μ and 535 m μ as well as with a Hilger "Spekker Absorptiometer" using a green filter.

The results of the last thirty one analyses were examined statistically as shown in Table IV.

From the figures in Table IV it is clear that fairly good results can be obtained using an ordinary photo-electric colorimeter with a green light filter. It should be noted, however, that considerably more difficulty was experienced in plotting standard curves using this instrument. The plot is not a straight line as is the case when a spectrophotometer is used. Moreover the readings are affected more markedly by changes in the concentration of excess dithizone. Therefore, due to the gradual deterioration of the dithizone reagent solution, standards must be checked more often.

The difference in precision of the two sets of results obtained with the Cenco Sheard Spectrophotometer justified the decision to make photometric measurements at 520 m μ , rather than at 535 m μ . The effective band width used was approximately 12 m μ . (i.e. 514-526 m μ for the 520 m μ setting).

(The improved accuracy which was obtained later using the Beckman D. U. Spectrophotometer is partly attributable to the fact that it was possible to use an effective band width of only 1 m μ with this instrument).

TABLE IV./.

TABLE IV.

No. (n)	SPEKKER.		CENCO AT 535 mμ.		CENCO AT 520 mμ.	
	ppm. Zn.	(y-m) ²	ppm. Zn.	(y-m) ²	ppm. Zn.	(y-m) ²
1	42.2	0.00	42.2	0.00	42.0	0.25
2	42.4	0.04	43.2	0.64	42.9	0.16
3	40.5	2.89	41.8	0.36	41.7	0.64
4	41.8	0.16	41.7	0.49	41.3	1.44
5	40.4	3.24	42.9	0.25	42.6	0.01
6	41.8	0.16	42.7	0.09	42.0	0.25
7	42.5	0.09	43.6	1.44	42.8	0.09
8	41.1	1.21	43.0	0.36	43.2	0.49
9	42.6	0.16	42.7	0.09	42.8	0.09
10	42.7	0.25	42.9	0.25	42.8	0.09
11	43.0	0.64	43.0	0.36	43.3	0.64
12	42.6	0.16	42.0	0.16	42.7	0.04
13	42.5	0.09	42.1	0.09	42.7	0.04
14	44.2	4.00	44.0	2.56	43.8	1.69
15	43.3	1.21	42.7	0.09	43.4	0.81
16	41.6	0.36	41.9	0.25	42.3	0.04
17	42.0	0.04	41.8	0.36	42.0	0.25
18	40.6	2.56	40.7	2.89	41.2	1.69
19	42.2	0.00	41.7	0.49	41.9	0.36
20	40.9	1.69	41.2	1.44	41.8	0.49
21	42.6	0.16	41.8	0.36	42.0	0.25
22	43.3	1.21	43.3	0.81	43.3	0.64
23	43.0	0.64	41.8	0.36	42.4	0.01
24	43.7	2.25	42.2	0.04	42.9	0.16
25	43.0	0.64	42.5	0.01	43.3	0.64
26	42.7	0.25	43.5	1.21	43.0	0.25
27	41.1	1.21	41.0	1.96	41.0	2.25
28	41.0	1.44	41.0	1.96	42.3	0.04
29	44.0	3.24	44.5	4.41	44.7	4.84
30	41.1	1.21	41.3	1.21	41.8	0.49
31	42.0	0.04	42.9	0.25	43.1	0.36
TOTAL.	1308.4	31.24	1313.0	25.24	1319.0	19.49
MEAN.	42.2		42.4		42.5	

STD. DEV. 1.02 ppm. 0.92 ppm. 0.81 ppm.
 % STD. DEV. 2.41 %. 2.16 %. 1.90 %.

6. EXPERIMENTAL etc./.....

6. EXPERIMENTAL STUDIES ON THE USE OF DI-BETA-NAPHTHYL THIOCARBAZONE.

(a) TRANSMISSION CURVES OF CARBON TETRACHLORIDE SOLUTIONS OF DI-BETA-NAPHTHYL THIOCARBAZONE AND ITS ZINC COMPLEX.

Cholak and his co-authors (10) published transmission curves for solutions, in chloroform, of di-beta-naphthyl thiocarbazon (DN) and its zinc complex and, on the basis of these curves, they recommended that, in the DN-colorimetric method, photometric readings be taken at 550 m μ or 650 m μ . As however, no previous attempt to use this reagent in carbon tetrachloride has been reported in the literature, it was necessary, in case of any differences due to the solvent, to plot the transmission curves for the carbon tetrachloride solutions.

(i) Zinc Complex.

10 ml. of di-beta-naphthyl thiocarbazon solution (about .005%) were shaken vigorously with 10 ml. of 5 ppm. zinc solution in 40 ml. of an ammonia-ammonium citrate solution (pH 8.5). The carbon tetrachloride layer was then run into a second separatory funnel to which 5 ml. more of the zinc solution and 40 ml. of the ammonia-ammonium citrate solution were added. This was shaken vigorously for two minutes and finally, the carbon tetrachloride phase was allowed to separate and was run into a 50 ml. volumetric flask and made to the mark. In this way an excess of zinc was ensured and as much as possible of the DN was converted to the zinc complex.

(ii)/.....

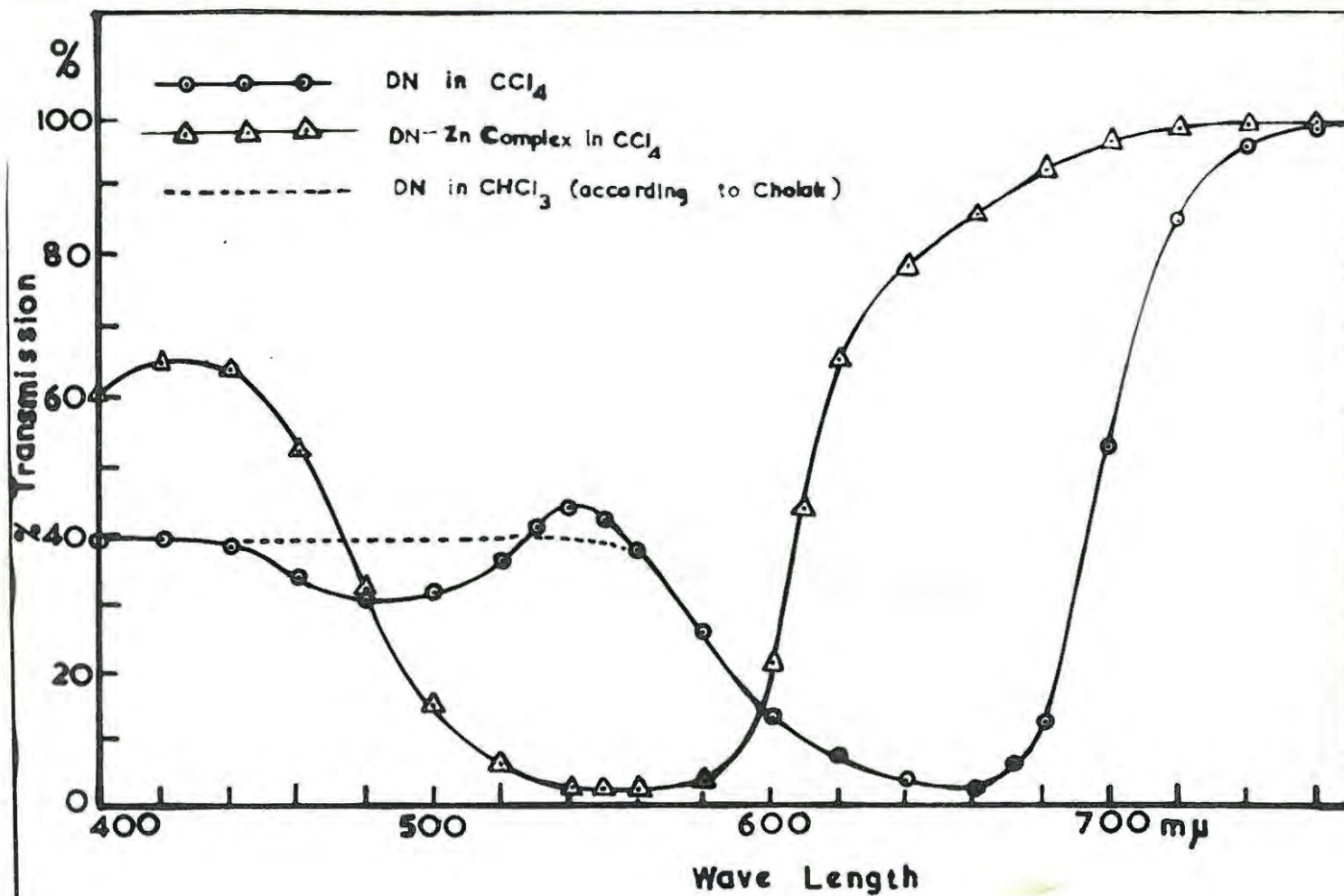


FIG. 6.

TRANSMISSION CURVES OF DI-β-NAPHTHLYTHIOCARBAZONE AND ITS ZINC COMPLEX

(ii) Di-Beta-Naphthyl Thiocarbazone.

10 ml. of the DN solution were diluted to 50 ml. with pure carbon tetrachloride.

Transmission measurements for each solution were made between 400 and 760 μ employing a Beckman D.U. Spectrophotometer.

The results of this investigation are shown in the graph (Figure 6).

The curves obtained agreed almost exactly with those obtained by Cholak et al. (17) for the corresponding chloroform solutions, apart from the slight minimum and maximum which the DN curve shows at 480 μ and 540 μ . It was thought that this might be due to the difference in the solvent, but on repeating the measurements with a chloroform solution, a similar result was obtained.

This difference may be due to either:

- (a) Impurities in the reagent or
- (b) Cholak and his co-workers using a wider effective bandwidth in making transmission measurements.

Selection of Wave Length for Transmission Measurements.

It was decided that transmission measurements should be made at 540 μ . This wave length was selected in preference to 660 μ (where the separation of the curves is greatest) because:

- (a) This is a more suitable wave length for simple instruments, being in the middle of the visible region.
- (b)/.....

- (b) More importance is attached in this case to the concentration of the zinc complex rather than to the concentration of excess reagent which is dependent on the rate of deterioration of the solution and on the accuracy with which the volumes used are measured.

(b) THE INFLUENCE OF pH ON THE EFFICIENCY OF EXTRACTION OF ZINC WITH DI - BETA - NAPHTHYLTHIOCARBAZONE.

Cholak and his co-workers (10) in investigating the effect of pH on the extraction of zinc with chloroform solutions of DN (150% excess) found that a 100% extraction was obtained between pH 8 and 11. It was decided to investigate the relationship for a carbon tetrachloride solution of DN.

For this investigation much the same procedure as for dithizone was followed. The citrate concentration used was, however, about 0.05M, as recommended by Martin (20).

After a preliminary/.....

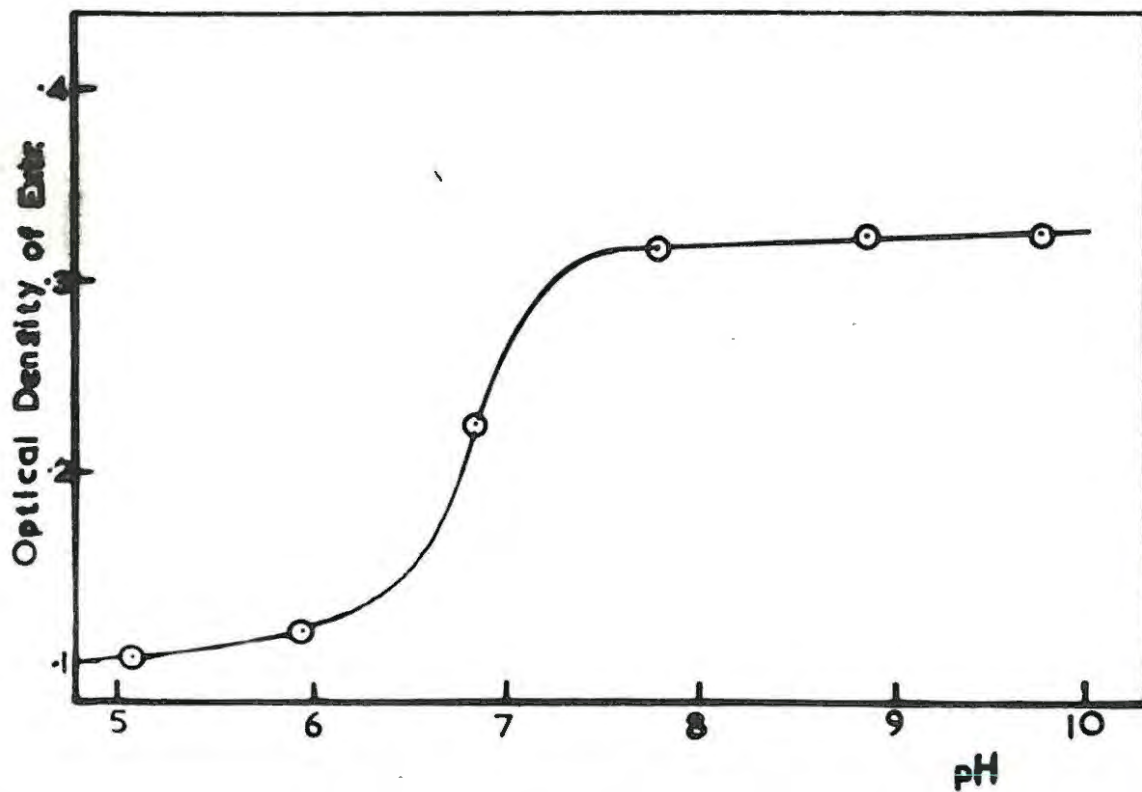


FIG. 7.

THE INFLUENCE OF pH ON THE EXTRACTION OF
ZINC WITH DI-BETA-NAPHTHYL-THIOCARBAZONE.

After a preliminary extraction of the citrate buffer to remove impurities, 5 ml. of 2 ppm zinc solution was added, and the solution extracted by shaking for two minutes with 0.003% DN. The carbon tetrachloride layer was then run into a 50 ml volumetric flask, the remaining traces being rinsed down with pure carbon tetrachloride. The solution was diluted to the mark and its optical density measured as before.

NOTE: As DN is not soluble in alkaline aqueous media, it is not possible to remove excess reagent and the method is a true "mix-colour" one.

The results obtained were in agreement with those of Cholak, in that maximum extraction was attained between pH 7.5 and 8, and maintained until pH 10. (see Figure 7).

(c) THE SOLUBILITY OF DI-BETA-NAPHTHYLTHIOCARBAZONE.

One of the chief disadvantages of DN when compared with Dz is its smaller solubility. This is particularly marked when carbon tetrachloride is used as solvent. As, however, the literature contains no reference to the use of this reagent in carbon tetrachloride, it was considered worthwhile to investigate the possibilities of a DN method using carbon tetrachloride as solvent.

After one or two/....

After one or two trials it was found that 0.03g/l. was the highest concentration of DN which could be readily obtained. Calculating on the assumption that the reaction of DN with metals is similar to that of dithizone, 10 ml. of this solution should be sufficient to complex more than 25 μ g of zinc. It was found, however, that 10 μ g of zinc was the maximum amount that could safely be extracted with 10 ml. of this solution at a favourable pH.

NOTE: Cholak recommends 0.02 g/l. for 0 - 5 μ g Zn.

(d) PRACTICAL CONSIDERATIONS.

(i) Citrate Concentration.

Martin (20) suggested that the high concentration of citrate used by Cholak and his co-workers (0.26M) was likely to reduce the extraction efficiency. As there is less need for careful pH control when DN is used, there does not seem to be any good argument in favour of using a great deal more citrate than is required to complex all the iron and aluminium which would otherwise precipitate. It was therefore decided to use an 0.05M solution of citrate. (Martin used 0.048M).

(ii)/.....

(ii) Concentration of Acid Used in the Second Stage of the Analysis.

To transfer the zinc back to the aqueous phase after the initial extraction, Martin used 20 ml. of 0.5M hydrochloric acid, while Cholak used 50 ml. of 0.2N acid. In view of the fact that the greater the acid concentration at this stage, the more ammonia must be used to bring the pH to right value for the final extraction, it was decided to test the suitability of a range of acid concentrations in the manner described below.

The first step of the analysis was carried out in the usual manner in triplicate, 10 μ g of zinc being extracted from approximately 0.05M ammonium citrate at pH 9, by shaking with 10 ml. of DN reagent. Each of the extracts was then shaken for two minutes with 40 ml of an acid solution, the concentration of acid used being 0.05N in the first case, 0.1N in the second case and 0.2N in the third.

The final extraction with DN was carried out after the addition of 40 ml. of 0.05M citrate solution, containing a sufficient concentration of ammonia to bring the pH to approximately 9. (This concentration was determined beforehand).

The final results of this experiment (see Table V) showed no difference between the use of 0.2N and 0.1N acid, but the results for the analyses, in which 0.05N acid was used, was in each case slightly lower.

TABLE V: The Effect of the Acid Concentration in the Second Extraction on the results of the DN-method.

Expt.No.	Optical Density Reading.		
	0.05N	0.1N	0.2N
1	0.247	0.321	0.319
2	0.294	0.315	0.318

(iii) Stock Buffer Solutions.

For convenience in routine analytical work, two stock buffer solutions were prepared analogous to those used in the Cowling and Miller method:-

Solution A'. 400 ml. of 0.5M ammonium citrate plus 300 ml. 1.0N NH_4OH . This mixture was extracted repeatedly with 100 ml. portions of DN reagent, until all traces of heavy metals had been removed, and then it was diluted to 4 litres.

Solution B'. A mixture of 750 ml. N ammonia solution plus 450 ml of 0.5M ammonium citrate was extracted with DN as above, and then made to 4 litres. This solution was mixed 9 to 1 with carbamate solution just before use, as in the method of Cowling and Miller.

(e)/.....

(e) REPRODUCIBILITY TESTS ON THE METHOD.

The next step was to test the reproducibility by carrying out repeated analyses on standards. The first results of the analysis of a set of standards, containing from 2 to 10 μg of zinc, gave a set of points lying on a straight line (see Figure 8).

Readings obtained using the "Spekker Absorptio-
meter, indicated that moderately good results might be expected if this instrument were used for the analysis. (See graph, Figure 8).

Later results, however, though again giving a good standard curve were in complete disagreement with the first set of results, and it was found to be impossible to get good agreement between sets of standards. Table VI shows two typical sets of standard determinations carried out on consecutive days.

TABLE VI: Results of Standard Analyses by the DN-method.

Zn present.	<u>Beckman Reading.</u>		<u>Spekker Reading.</u>	
	Analysis 1.	Analysis 2.	Analysis 1.	Analysis 2.
10 μg	470	.380	.299	.249
8	416	.340	.272	.230
6	358	.302	.247	.210
4	312	.259	.219	.191
2	265	.222	.199	.172

These/.....

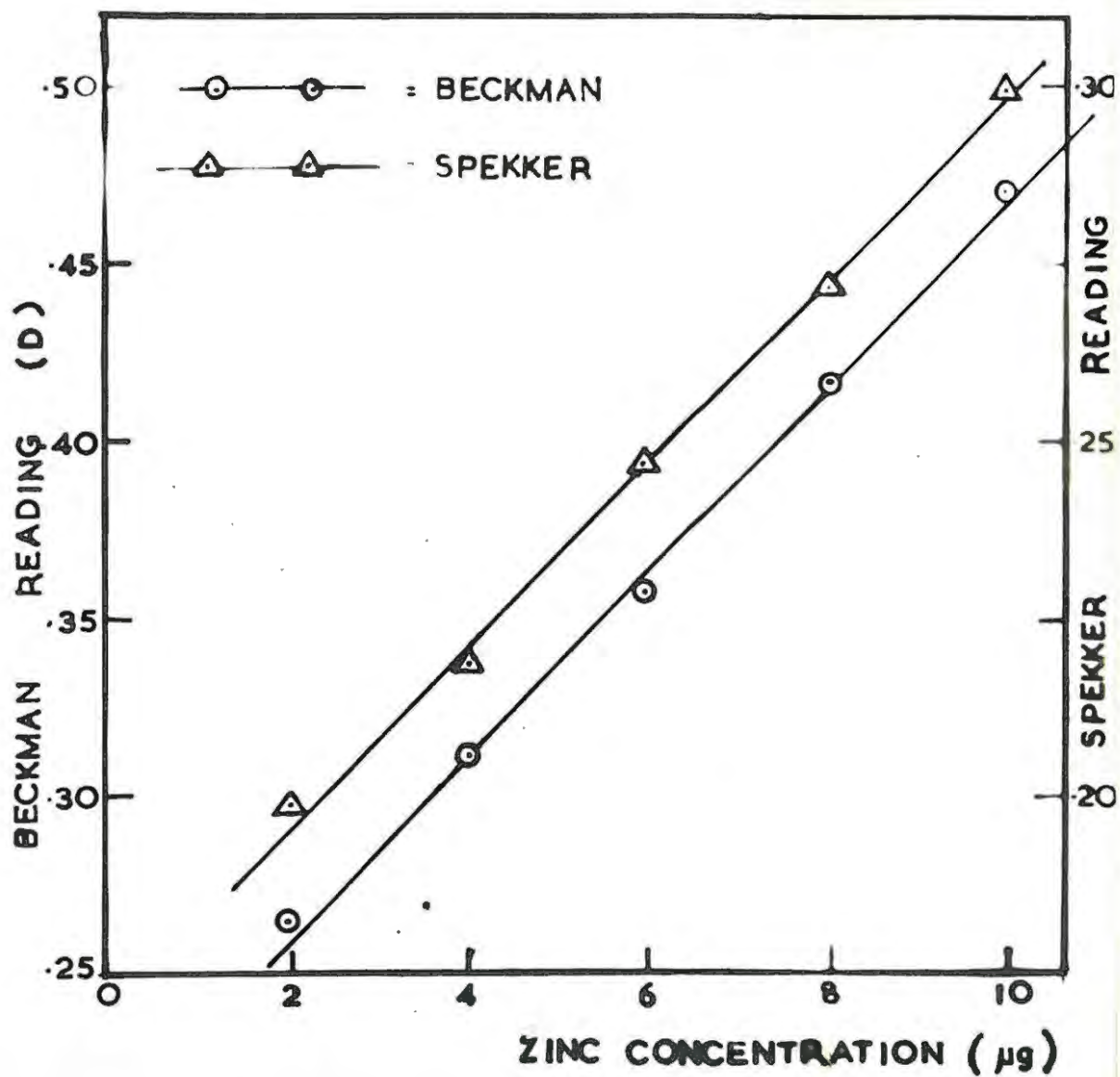


FIG. 8.

STANDARD CURVES OBTAINED BY
THE DN-COLORIMETRIC METHOD.

These discrepancies were at first attributed to the different lengths of time taken for the last stages of the analysis, but the optical density of a set of Zn extract solutions left standing for fourteen hours showed only a small change.

Each step of the analysis was checked to ascertain whether variable amounts of zinc were being lost in the course of the analysis, but experiments showed only a very small uniform loss between the first and the third stage.

Standardisation of the time spent on each step of the analysis by doing each determination separately, failed to produce the desired result.

The final conclusion reached was that the differences must be due to the rapid deterioration of the stock DN reagent solution.

An attempt to correct for this by a blank determination was unsuccessful.

It was finally decided to abandon the attempt to develop a method of analysis using di-beta-naphthylthiocarbazone in carbon tetrachloride.

PART II./.....

P A R T II.

THE POLAROGRAPHIC DETERMINATION OF ZINC IN
SOILS AND PLANTS.

An investigation of the methods for the determination of zinc in soils and plants would not be complete without a study of the polarographic methods. While it was felt to be unlikely that greater precision would be attained with the polarograph, it was possible that this technique might show other advantages over the colorimetric method.

Another reason for undertaking this part of the work was that, though the precision of any given method may be tested satisfactorily by carrying out a sufficient number of analyses, its absolute accuracy can only readily be tested by comparison of its results with those of a completely independent method.

1. REVIEW etc./.....

1. REVIEW OF PREVIOUS WORK.

(a) GENERAL REVIEW OF PUBLISHED WORK.

The methods described by Koltoff and Lingane (26) in their comprehensive book on Polarography are those of Stout, Levy and Williams (27) and Reed and Cummings (28). In both of these methods a supporting electrolyte composed of potassium thiocyanate and ammonium acetate-acetic acid buffer (pH 4.6) was used in the final stage of the analysis. Stout and his co-workers used dithizone to separate the zinc and other heavy metals from the major constituents of the sample, while Reed and Cummings employed ammonia to separate iron and aluminium at pH 4.5. In the latter method, although it has the advantage of simplicity, there is a danger of losing trace elements by co-precipitation.

In 1942 Allan Walkley (21) published a method very similar to that of Stout and his co-workers. After extraction of the zinc with dithizone in chloroform and the subsequent destruction of the dithizonates, the extract was taken up in a supporting electrolyte of ammonium chloride and potassium thiocyanate at pH 7.

Futoshi Takazawa and G.D. Sherman (29) claimed an accuracy of about 2%, over a wide range of zinc concentrations, for their method, in which the sample solution was prepared by sodium carbonate fusion, the zinc extracted with dithizone in chloroform and then transferred to an aqueous acid solution which was then electrolysed.

In 1951/.....

In 1951 R.G. Menzel and M.L Jackson (30) published a method in which, after extraction with dithizone in carbon tetrachloride, the concentration of zinc was determined polarographically in a supporting electrolyte containing sodium sulphite, ammonia and gelatin. The waves due to zinc and cobalt coincide, but the cobalt was considered negligible in comparison with the zinc, which is usually the case in biological materials.

G.B. Jones (31) recommended the use of a biphthalate supporting electrolyte after the extraction of zinc and copper with dithizone. This procedure has certain advantages for the copper determination, namely that the reduction of the cupric ion to the metal takes place in one step, giving one large wave for this metal instead of the usual two obtained in other supporting electrolytes.

Cholak (10) and Martin (20) have recommended the use of di-beta-naphthylthiocarbazonone for the extraction of the zinc, but otherwise the methods which these authors describe are very similar to that of Walkley.

In a recent publication Jones (32) has described a method for the determination of zinc and manganese in biological material in which iron is first removed as the cup-ferron complex by extraction with chloroform. The zinc and manganese are then extracted with sodium diethyl dithiocarbamate and chloroform. After evaporation of the chloroform and destruction of the complexes, the residue is/.....

due is treated with hydrochloric acid and finally taken up in lithium chloride solution for the electrolysis.

Of these methods, that of Reed and Cummings (28) was first selected for study in view of its greater simplicity and the fact that it is in no way related to the colorimetric method. The procedure used by these workers is therefore given in greater detail.

(b) THE METHOD OF REED AND CUMMINGS.

0.5 to 2 g. of plant material were ashed at 450°C, evaporated to dryness with concentrated hydrochloric acid and finally taken up in N hydrochloric acid. The pH of this solution was adjusted to between 4 and 5 (pH meter) with dilute ammonia solution, filtered, and the filtrate evaporated to dryness. The residue was taken up in a solution which was 0.1M with respect to ammonium acetate (pH 4.6) and 0.025M with respect to potassium thiocyanate.

Oxygen was removed from the solution by bubbling pure nitrogen through the cell and the zinc determined polarographically with a Heyrovsky Polarograph. The dropping mercury electrode was constructed according to Koltoff and Lingane (26).

A constant dropping rate of 2.0 to 2.6 seconds per drop was found most satisfactory.

To determine the half wave potential, the anode potential was measured against a saturated calomel

electrode./.....

electrode. The anode potential, under these conditions, only varied slightly.

Adjustment of the pH to 4-5 removes from solution practically all the aluminium and ferric iron. Although the alkali metals and most of the other constituents are not removed, zinc may be determined polarographically in their presence.

The authors showed that no interference was caused by any of the anions or cations likely to be found in plant ash, even when present in concentrations much larger than commonly found.

Limits of the method, using a 1.0 g. sample of plant material were reported to be from 0.5% or greater to 0.0005% of zinc in the plant and the accuracy to be within $\pm 5\%$.

(c) CRITICISM OF PUBLISHED METHODS.

All these methods have a common disadvantage, in that a preliminary treatment of the soil or plant solution is required to separate interfering ions before the zinc, which is a minor constituent, can be determined. Of these interfering elements iron is the most important as it is usually present in relatively high concentrations particularly in the case of soil solutions.

Thus,/.....

Thus, in the method of Reed and Cummings, outlined above, more than half the total time spent on the analysis is taken by the preliminary separation, which may, moreover, cause serious errors due to co-precipitation.

In many cases use of the polarographic technique eliminates the need for preliminary separations, usually by the formation of complexes. In the course of the work for this thesis an attempt was therefore made to devise a new procedure which would eliminate the common disadvantage of the above methods.

Thus, although the procedure described by Reed and Cummings was used in developing the practical polarographic technique, at an early stage work was commenced on the development of the entirely new procedure depending on the complexing action of fluoride. (A full description of this method is given in Section 3).

2. EXPERIMENTAL etc./.....

2. EXPERIMENTAL STUDIES ON THE POLAROGRAPHIC METHOD.

(a) DESCRIPTION OF THE APPARATUS AND SET-UP.

(i) The Polarograph.

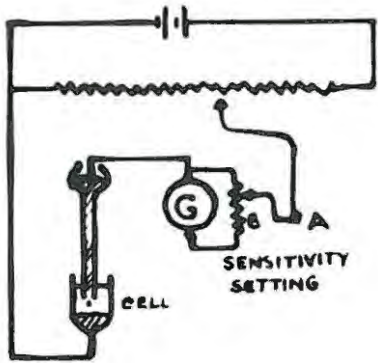
The instrument used was a Leybold Nachfolger polarograph which has been modified from time to time, particularly with a view to insuring good contacts on the axle of the potentiometer drum and at the moving contact. (See thesis of A.J. Eve (33)).

(ii) Charging Current Compensation.

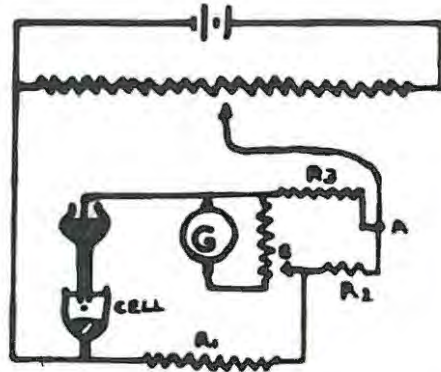
In most of the work it was necessary to operate at the maximum, or nearly the maximum sensitivity of the galvanometer in order to record measurable waves for the small concentrations of zinc present. Under these conditions the measurement of the wave height is rendered difficult, owing to the almost linear "charging" or "condenser current" which is superimposed on the normal current, due to the depolarisation of the mercury cathode.

Ilkovic and Semerano (34) have shown that this charging current can be compensated by means of a relatively simple electrical circuit based on the Wheatstone bridge. Three suitable resistances were therefore built into a small box and connected to the polarograph as indicated by these workers. (See diagram, Figure 9).

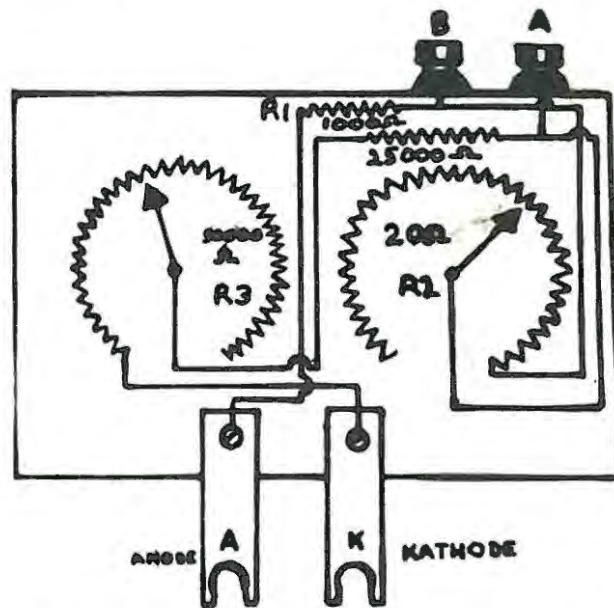
The current voltage curves obtained after this modification of the instrument were well defined even at the maximum galvanometer sensitivities. (iii)/.....



NORMAL CIRCUIT



WITH COMPENSATOR



CIRCUIT OF COMPENSATOR

FIG. 9.

CHARGING CURRENT COMPENSATION

(iii) Thermostat.

All measurements were made at 25.0°C, the electrolysis cells being immersed in a small thermostat bath (as described by A.J. Eve (33)) kept at that temperature.

(iv) Cells.

The cells used were of the type described by A.J. Eve (33). These had an internal pool anode. The potential of this anode was measured against a saturated calomel electrode, before and after recording a polarogram, when determining half wave potentials.

(b) OTHER PRACTICAL CONSIDERATIONS.

(i) Cathode Dropping Rate.

All measurements were carried out using a normal dropping mercury cathode, the drop rate of which was in the region of 3 seconds per drop.

(ii) Nitrogen.

Ordinary commercial nitrogen, without further purification, was found to be satisfactory for the elimination of the oxygen in the solution before electrolysis.

(iii) Reagents.

All reagents used were of A.R. grade and were used without further purification except in the case of/.....

ammonia, C.P. ammonia being distilled for use as described in Part I, Section 4.

(c) THE USE OF FLUORIDE AS SUPPORTING ELECTROLYTE.

Von Stackelberg and von Freyhold (35) reported that ferric iron produces a reduction wave in fluoride medium (pH not specified) and that the half wave potential is constant at -1.36 volts (vs. S.C.E.) when the concentration of potassium fluoride is varied between 0.04 and 0.8M. On the other hand, West and Dean using a 1M sodium fluoride supporting electrolyte of pH between 4 and 7 and containing 0.004% gelatin claimed that no reduction wave for the ferri-fluoride complex was observable below the discharge potential of the sodium ion. (Zinc gives a wave at -1.17 v.) A.J. Eve (33) completely eliminated the wave due to ferric iron in a potassium tartrate solution by the addition of small quantities of sodium fluoride.

To test the use of fluoride a simple experiment was carried out as follows:-

A solution, 1M with respect to sodium fluoride and 0.05M with respect to KCNS, was prepared. A 2% Fe solution was prepared using A.R. FeCl_3 .

5 ml of the sodium fluoride-potassium thiocyanate solution were pipetted into the polarograph cell and about 4 ml. of water added. The polarogram was recorded.

1 ml. of the ferric solution was added and the polarogram was again recorded. No wave/.....

No wave due to iron was observed.

The experiment was repeated with 20 μg of zinc in the solution whereupon the zinc wave was found to be recorded quite satisfactorily.

The complexing of the iron by fluoride produced a fine white crystalline precipitate, which, however, did not appear to interfere in any way with the determination.

(d) THE SUPPRESSIVE EFFECT OF FLUORIDE ON THE ZINC WAVE.

At first some difficulty was experienced in obtaining good standard analysis curves and agreement between sets of standards was particularly hard to obtain. As it had been noticed that concentrated fluoride solutions suppressed the cobalt wave, it was thought possible that this difficulty might be due to the fluoride suppressing the zinc wave. In the analysis up to this stage, a concentration of fluoride of approximately 1M had been used, but no attempt had been made to control the fluoride concentration accurately (1 g. \pm 0.1 g. added to 25 ml. of solution), as it was realised that the final concentration in an analysis would be dependent on the quantity of iron in the sample.

A preliminary test showed that the zinc wave-height was considerably increased when the fluoride concentration was reduced by half. Consequently a full investigation was carried out as follows:-

/.....

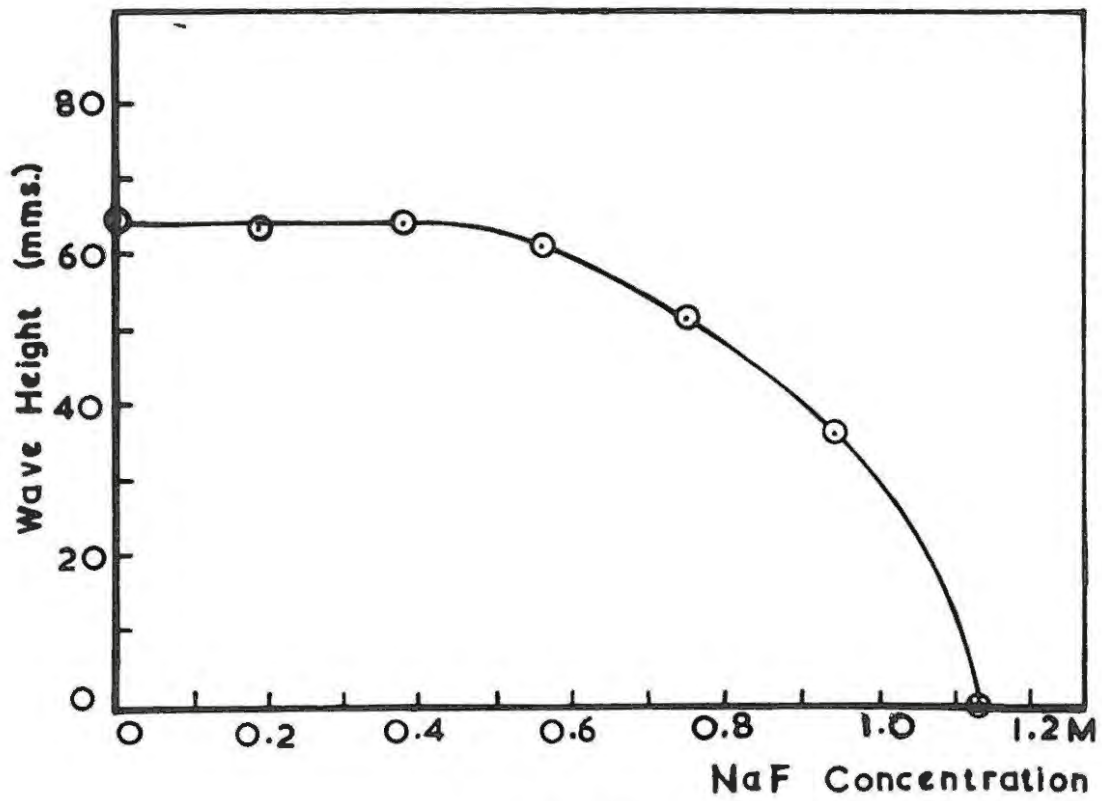


FIG. 10.

THE INFLUENCE OF FLUORIDE CONCENTRATION
ON THE ZINC WAVE

A measured quantity of zinc standard solution was run from a micro burette into a series of 25 ml. volumetric flasks. To each a different weighed quantity of fluoride was then added and the polarograms recorded. The results are shown in the form of a graph in Figure 10. The plot of wave height against fluoride concentration made it evident that, if consistent results were to be obtained, fluoride concentrations of greater than 0.5 molar could not be used, as, although the wave height varied by less than 1 mm. between zero and 0.5M fluoride concentration, the suppressive action of the fluoride was increasingly pronounced between 0.5M and 1.2M.

(e) THE EFFECT OF pH.

In order that the iron should be complexed by the fluoride it was found necessary that the solution should be fairly acid, before the addition of the fluoride (i.e. pH about 2). This is in agreement with an observation reported in a recent paper by Shoemaker (37). However, it was found necessary to neutralise the solution accurately immediately after the fluoride had been added because of two other effects which were observed, namely:-

(i)/.....

(i) If the solution remained acid (pH less than about 5) a rapidly growing wave was found to occur at -0.5 v. (vs. S.C.E.). This wave was found to be produced in the presence of fluoride and acid alone. It was found moreover, that the wave disappeared immediately when the contents of the cell were stirred up or when the cathode was moved.

By using a separate anode it was proved that the condition was independent of any effect at or near the mercury pool. (It was initially suspected that the phenomenon was due to the settling out of the fluoride complex on the anode, but this precipitate was, in any case, not obtained in the absence of iron).

(ii) If the solution was not accurately neutralised (to pH 6 - 7), a wave was found to occur, starting at about -1.3 v. (vs. S.C.E.), which interfered somewhat with the measurement of the zinc wave, although measurement was still possible, but with somewhat reduced accuracy.

In view of the observations of von Stackelberg and von Freyhold (35) this wave was attributed to iron in the sample but no attempt was made to verify this. In a very recent publication by Rulfs and Stoner (38) it was reported that a wave at -1.36 v. was produced in solutions containing fluoride alone, at pH between 5.5 and 6.5. These workers also reported the occurrence of a wave at -0.49 v. due to the reduction of iron in the fluoride solution.

If the/.....

If the original solution is very acid (i.e. pH very much less than 2) when fluoride is added, the fluoride appears to be immediately decomposed and no polarograms can be recorded for such solutions, even if they are neutralised immediately after the addition of fluoride.

(f) THE CONCENTRATION OF POTASSIUM THIOCYANATE AND THE SEPARATION OF WAVES OF INTERFERING ELEMENTS.

It has become fairly common practice to use potassium thiocyanate solutions in the polarographic determination of zinc. In solutions of thiocyanate the nickel ion is complexed and the wave due to nickel is well separated from the zinc wave.

As thiocyanate affects the half wave potential for the reduction of Co too, it was necessary to determine the thiocyanate concentration which would produce the best separation of the waves for nickel, zinc and cobalt. This was done in the following manner:-

A solution, 0.5M with respect to sodium fluoride containing 3 ppm. zinc, 3 ppm. nickel and 2 ppm. cobalt, was prepared. 10 ml. of this solution were pipetted into the polarograph cell and 0.2 ml. of 0.1% agar added. The polarogram of the solution was then recorded. Successive additions of 0.5% KCNS were then made and the polarogram recorded after each addition.

The/.....

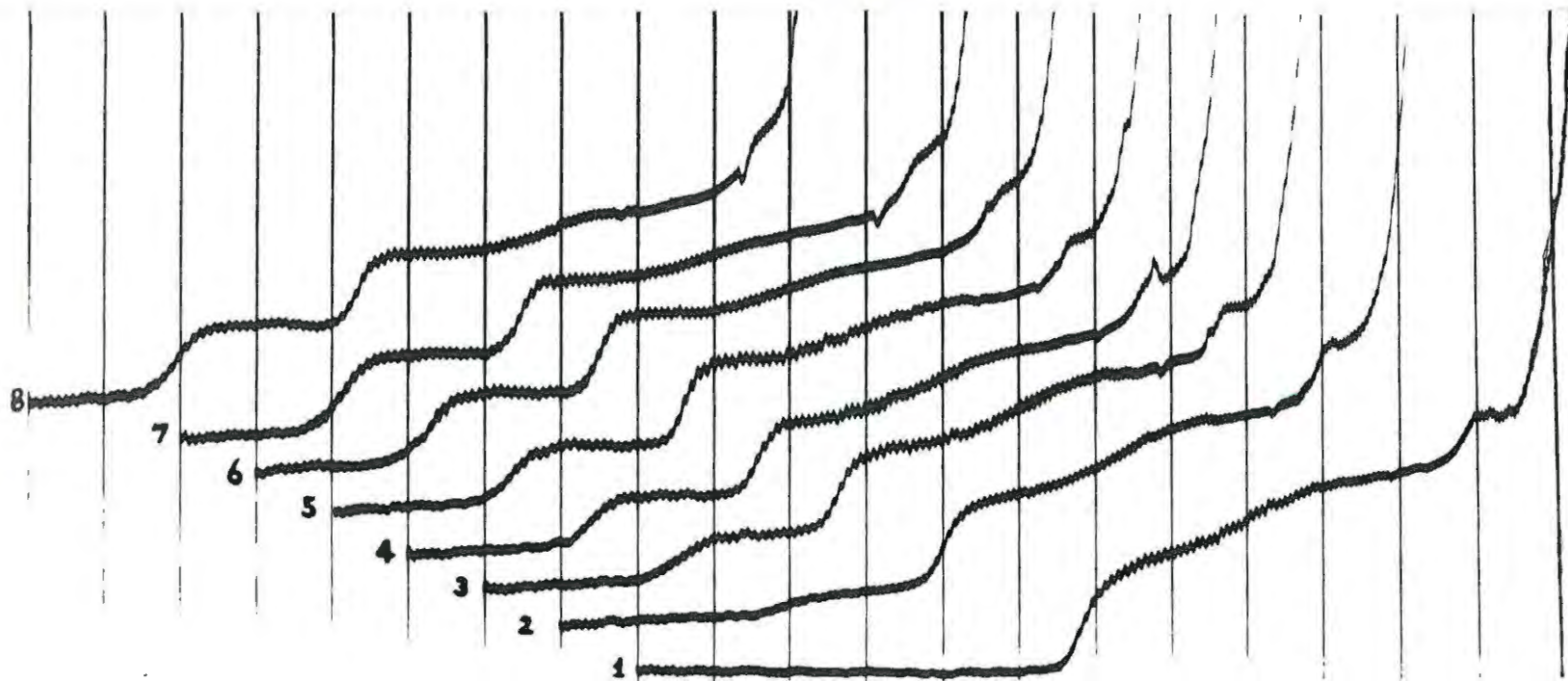


FIG. 11.

POLAROGRAMS SHOWING THE EFFECT OF VARYING KCNS CONCENTRATION
ON THE SEPARATION OF THE WAVES OF Ni, Zn AND Co.

The polarograms obtained are shown in Figure 11.

Results of a quantitative examination of the polarograms are shown in Table VII.

In view of the volume additions, and the fact that the galvanometer was used at only a fifth of its full sensitivity, these results cannot be regarded as strictly quantitative. However, on inspection of the results and the polarograms, it was decided that a concentration between 0.45 and 0.6% was likely to prove most suitable.

0.6% potassium thiocyanate was used in all the analyses by this method.

TABLE VII:

Polarogram No.	Vol. KCNS Added.	∴ % KCNS.	Wave Ht. Ni.	Wave Ht. Zn.	Wave Ht. Co.
1	0.0	0.0	-	-	6 mm.
2	0.2	0.098	3 mm.	10 mm.	6
3	0.4	0.19	5	10	6
4	0.6	0.28	6	9.5	6
5	0.8	0.37	7	9	6
6	1.0	0.45	8	9	6
7	1.4	0.61	8	9.5	6
8	1.8	0.76	8	9	5

(g)/.....

(g) THE COBALT WAVE.

The cobalt wave was observed (especially in the presence of gelatin) to be composed of a small "prewave" followed by a larger wave representing the main reduction of the Co^{2+} ion. This prewave was thought, at first, to be similar to that observed by Brdicka while working with cobaltous chloride solutions, and attributed by him to a basic product of the hydrolysis of the cobaltous ion. He caused this prewave to disappear by the addition of acid to bring down the pH slightly. Similar treatment by the author failed to have any effect on the prewave obtained in sodium fluoride - potassium thiocyanate solutions and it was eventually attributed to a partial complexing of the cobalt by the thiocyanate.

(h) MAXIMUM SUPPRESSOR.

At first gelatin (0.004% in final solution) was used as maximum suppressor. It was observed, however, that the gelatin caused a slight shift of the cobalt wave to a more positive potential (about 0.05 v. more positive). In addition the cobalt prewave (described above) appeared less pronounced when gelatin was not used.

The reason for this is not clear, but it may have been due to some impurity in the gelatin.

Because of/.....

Because of the high sensitivity required for this work, maximum suppressors which are reduced at the dropping mercury cathode were found to be unsuitable, even when used in small concentrations.

Eventually 0.002% agar was found to be quite satisfactory.

(i) THE USE OF BROMO-THYMOL BLUE.

It has been shown that it was necessary to neutralise the solution accurately before analysis. Bromo-thymol blue was selected as indicator as it has the most suitable pH range (yellow-blue pH 6.0 - 7.6). Checks made by pH meter on solutions, which were neutralised using bromo-thymol blue as indicator, showed the pH of the solutions in all cases to be between 6 and 7.

Bromo-thymol blue, as an experiment with a comparatively concentrated solution showed, is reduced at the dropping mercury cathode at about -0.85 v. (vs. S.C.E.), but, for the very small concentration used in the analysis, it was found that, even with the galvanometer at its full sensitivity, the wave produced was negligible.

3. DESCRIPTION etc./.....

3. DESCRIPTION OF THE NEW POLAROGRAPHIC METHOD.

As a result of the observations described above, the following method for the determination of zinc in plants and soils was proposed.

Apparatus:

The apparatus used in this method was as described in Part II, section 2 with one addition. The method requires that a weighed quantity solid sodium fluoride be added to a 25 ml volumetric flask. For this purpose a special weighing boat was designed. Made of pyrex glass, it consisted of a flat boat with a funnel-like opening at one end. The weighing boat is illustrated in Figure 12.

Reagents:

- (i) Sodium Fluoride: The A.R. salt was used.
- (ii) 15% KCNS: A solution of this strength was prepared from A.R. KCNS and zinc-free water.
- (iii) 0.1% Bromo-thymol Blue: 0.1 g. of the compound was dissolved in the minimum quantity of hot absolute alcohol and then diluted to 100 ml with Zn-free water.
- (iv) Redistilled Ammonia: This was prepared as described in Part I, section 4. *pag 52 / W*
- (v) 0.2% Agar Solution: 0.2 g. of agar-agar was dissolved in 100 ml of warm zinc-free water.
- (vi) Zinc Standard Solution: Prepare as described in Part I, section 3(a).

Procedure:/.....

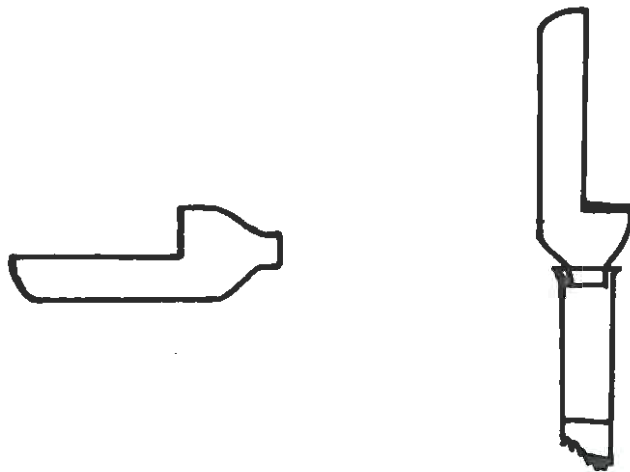


FIG: 12.

WEIGHING BOAT FOR THE ADDITION
OF FLUORIDE TO VOLUMETRIC FLASK

Procedure:

Pipette out an aliquot of the soil or plant solution containing between 10 and 100 μg of zinc into a 25 ml. volumetric flask. (If the solution is very acid raise the pH to about 2 by the addition of redistilled ammonia). Add exactly 0.50 grams of A.R. sodium fluoride and mix thoroughly. At this stage the yellow colour of the solution due to iron disappears and a fine white precipitate of the iron fluoride complex begins to separate. Add 1 ml. of 15% potassium thiocyanate followed by one drop of 0.1% bromo-thymol blue. Shake and carefully add redistilled ammonia from a burette until the solution shows a faint, permanent blue-green colour (pH 6-7).

Next add 0.2 ml. of a 0.2% agar solution, dilute to the mark and mix thoroughly.

Use part of the solution to rinse the polarograph cell and pour the remainder (about 10 ml.) into the cell. After the contents have reached the temperature of the thermostat (25°C), record the polarogram from 0.8 to 1.2 volts.

Measure the height of the zinc wave and obtain the zinc concentration from a standard calibration curve.

Standard Curve:

This is prepared by running from 2 to 10 ml. of a 10 ppm, standard solution of zinc from a micro burette into each of a series of 25 ml. volumetric flasks and proceeding as described above. Wave heights in millimetres are plotted against concentration of zinc.

4. EXPERIMENTAL STUDIES ON THE APPLICABILITY OF THE NEW METHOD.

(a) THE INFLUENCE OF FOREIGN IONS.

(i) Half Wave Potentials.

The half wave potentials, with respect to the saturated calomel electrode, of all the ions likely to be encountered in plant or soil extracts were measured under the conditions of the analysis i.e. in neutral solution (pH 6-7), 0.5M with respect to sodium fluoride and 0.06M with respect to potassium thiocyanate (0.6%). The results are shown in Table VIII. For comparison, values obtained from the literature for the half wave potentials in solutions of potassium thiocyanate and sodium fluoride alone are given.

TABLE VIII:

Metal.	Half Wave Potentials (vs. S.C.E.)		
	NaF = 0.5M. KCNS = 0.06M. pH = 6 - 7.	NaF = 0.5M. pH = 4.6	KCNS = 1M.
Cu 1.	Near 0.0 v.	-0.003 v.	-0.02 v. (.1M)
2.	-0.45 v.		-0.39 v. (KCNS)
Ni	-0.68 v.	-1.12	-0.70 v.
Zn	-1.03 v.	-1.14	-1.06 (1.01 in) (0.1M KCNS)
Mn	-1.55 v.	-1.55	-1.55
Co	Indefinite (-1.3).	-1.38	-1.03
Fe	Not reduced.	Not reduced.	0

As the/.....

As the table shows, a better separation of the waves of the metals considered is obtained in the mixed electrolyte used than is obtained in either potassium thiocyanate or sodium fluoride solution alone.

(ii) Test of Interference from Foreign Ions.

In order to test the degree of interference from iron, copper, nickel, cobalt and manganese, under conditions similar to those likely to be encountered in plant and soil solutions, the following experiment was carried out.

A series of standards containing between 20 and 120 μg of zinc were analysed,

- (1) in the absence of any foreign ions;
- (2) in the presence of 0.08 g. iron, which represents an excess of this metal larger than normally encountered in soil solutions;
- (3) in the presence of 0.08 g. iron, 120 μg copper, 60 μg cobalt, 80 μg nickel, 2 mg. manganese.

Each solution in this final series was equivalent to a 2 g. sample of soil containing 4% iron, 1000 ppm manganese, 60 ppm copper, 30 ppm cobalt, 40 ppm nickel and 10 - 60 ppm zinc.

The results obtained are represented graphically in Figure 13.

From the results it is clear that no interference was caused by these ions, even under conditions such that their concentrations were relatively larger than is normally encountered in practice.

Of the other/.....

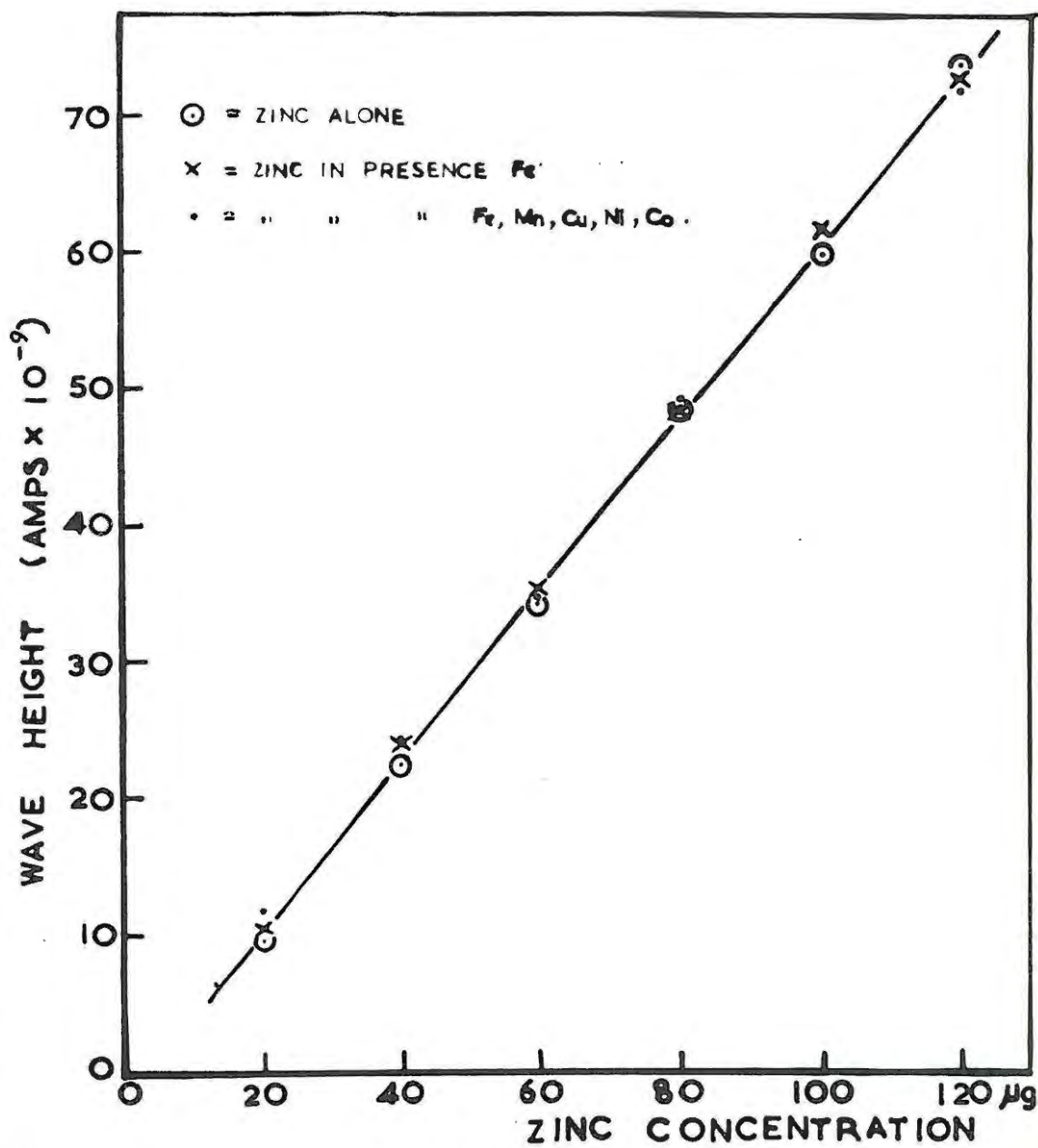


FIG. 13

ZINC DETN. IN THE PRESENCE OF FOREIGN IONS

Of the other elements likely to be found in plants and soils, aluminium, molybdenum, calcium, magnesium, potassium and sodium do not give waves under the conditions of the method and therefore do not interfere.

(b) THE POSSIBILITY OF ANALYSING FOR OTHER IONS SIMULTANEOUSLY.

Figure 14 shows a typical polarogram recorded during the analysis of an aliquot of the soil solution used for the statistical comparison with the colorimetric method.

As the polarogram shows, there is a possibility that copper, nickel, cobalt and manganese may be determined at the same time as zinc, though phosphate may interfere with the manganese determination.

A rapid test showed that 0.5 mg of manganese could readily be determined in the presence of .08 g. of iron and there is no doubt that lesser concentrations of manganese could be determined equally successfully by this method, in the presence of the same quantity of iron. Thus the method may possibly prove of some value in steel analysis.

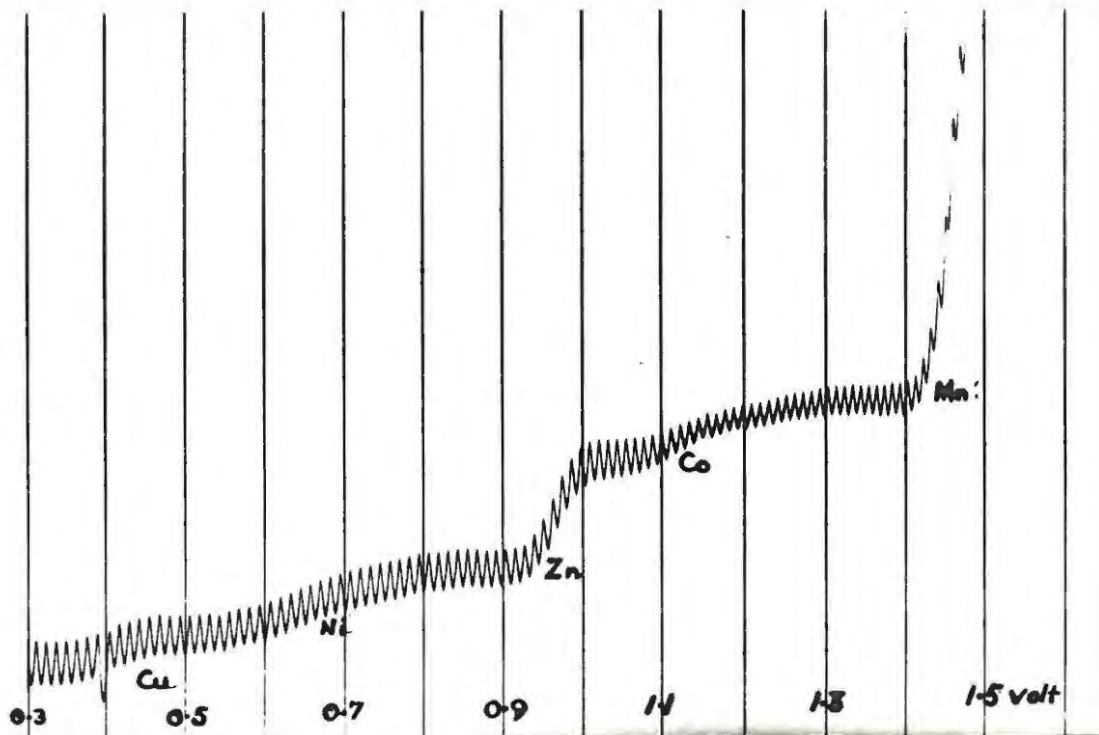


FIG. 14.

POLAROGRAM OBTAINED DURING ANALYSIS
OF SOIL SOLUTION.

P A R T III.

A STATISTICAL COMPARISON OF THE ACCURACY AND PRECISION
OF THE TWO METHODS.

1. EXPERIMENTAL PROCEDURE.

The modified colorimetric method and the new polarographic method were finally subjected to a rigorous statistical test in order to evaluate their relative merits, as well as to test the accuracy and precision of the two methods under routine laboratory conditions.

For this purpose, a standard soil solution was prepared by an exhaustive perchloric acid extraction technique. This ensured the presence of large concentrations of the foreign ions likely to be encountered in the analysis of biological materials.

A litre of soil solution was prepared from 100 g. of soil, as follows:-

A Grahamstown soil sample was ground to a fine powder in an agate mortar. Twenty separate 5 gram portions of the sample were weighed into 100 ml wide-mouthed erlenmeyer flasks, fitted with reflux covers (see Part IV, section 3(b)(ii), page 106). 15 ml of A.R. perchloric acid (S.G. 1.54) were added to each flask and the mixture heated at boiling point on a hot plate for one hour. The covers were then removed and the

contents/.....

contents of the flasks evaporated almost to dryness at a slightly higher temperature. The flasks were then allowed to cool and 50 ml. of zinc-free water and about 2 ml. of redistilled, constant boiling point hydrochloric acid added. The contents of the flask were then boiled under refluxing conditions for about 30 minutes and finally filtered hot through a Whatman No. 40 filter. The combined extracts from all the 5 g. samples were made up to 1 litre and stored in a polythene bottle.

The analyses were carried out as follows:-

(a) Colorimetric Analysis.

To 250 ml. of the soil solution 5 g. of A.R. ammonium citrate were added, the solution neutralised with re-distilled ammonia, made up to 500 ml in a volumetric flask and stored in a polythene bottle. 10 ml aliquots of this solution were analysed for zinc by the method described on page 51. A Beckman Model D.U. Spectrophotometer, set at a wave length of 520 m μ was used for the optical density measurements. Thirty analyses were carried out.

(b) Polarographic Analysis.

10 ml. aliquots of the soil solution were analysed for zinc by the method described on page 86.

2. STATISTICAL etc./.....

2. STATISTICAL TREATMENT OF THE RESULTS.

The results obtained by the two methods are shown in Table IX.

Comparison of the "Standard Error of Difference" and the difference between the mean values obtained by each method shows that there is no significant difference in the results obtained and, as the methods are completely unrelated, it indicates that there is no fundamental error in either method.

TABLE IX./.....

TABLE IX.

No. (n)	COLORIMETRIC ANALYSES.				POLAROGRAPHIC ANALYSES.			
	Mean Opt. Density.	Zinc ppm.	(y-m)	(y-m) ²	Mean Wave Ht.	Zinc ppm.	(y-m)	(y-m) ²
1	.467	30.6	.4	.16	15.0	32.5	1.0	1.0
2	.470	30.8	.2	.04	14.7	31.9	.4	.16
3	.481	31.7	.7	.49	14.1	30.8	.7	.49
4	.477	31.3	.3	.09	14.3	31.2	.3	.09
5	.478	31.4	.4	.16	14.9	32.3	.8	.64
6	.475	31.2	.2	.04	14.3	31.2	.3	.09
7	.479	31.5	.5	.25	13.8	30.3	1.2	1.44
8	.470	30.8	.2	.04	15.8	33.8	2.3	5.29
9	.487	32.1	1.1	1.21	15.5	33.4	1.9	3.61
10	.466	30.6	.4	.16	15.5	33.4	1.9	3.61
11	.465	30.4	.6	.36	15.8	33.8	2.3	5.29
12	.468	30.7	.3	.09	14.6	31.7	0.2	0.04
13	.464	30.3	0.7	.49	13.9	30.5	1.0	1.00
14	.470	30.8	0.2	.04	13.8	30.3	1.2	1.44
15	.469	30.8	.2	.04	13.2	29.3	2.2	4.84
16	.470	30.8	.2	.04	13.1	29.0	2.5	6.25
17	.472	31.0	0.0	0.00	15.3	33.0	1.5	2.25
18	.478	31.4	.4	.16	13.9	30.5	1.0	1.00
19	.469	30.8	.2	.04	14.0	30.7	0.8	0.64
20	.461	30.1	.9	.81	14.0	30.7	0.8	0.64
21	.467	30.6	.4	.16	14.9	32.3	0.8	.64
22	.468	30.7	.3	.09	14.8	32.1	0.6	.36
23	.481	31.7	.7	.49	14.0	30.7	0.8	.64
24	.472	31.0	0	0	14.1	30.8	0.7	.49
25	.482	31.8	0.8	.64	13.9	30.5	1.0	1.00
26	.471	30.9	.1	.01	15.3	33.0	1.5	2.25
27	.474	31.1	.1	.01	14.9	32.3	0.8	0.64
28	.484	31.9	.9	.81	15.3	33.0	1.5	2.25
29	.481	31.7	.7	.49	13.9	30.5	1.0	1.00
30	.465	30.4	.6	.36	14.3	31.1	0.4	.16
31					14.3	31.1	0.4	.16
32					14.7	31.8	0.3	.09
TOTAL MEAN.		930.9 31.0		7.77		1009.5 31.5		49.49

STAND. DEV. (6) = $\sqrt{\frac{(y-m)^2}{n-1}}$ = $\frac{0.518}{\text{ppm.}}$ = 1.26 ppm.

% STAND. " = $\frac{0.518}{31.0} \times 100$ = 1.67% = 4.01%

% STAND. ERROR. = $\frac{\delta}{\sqrt{n}} \times \frac{100}{M}$ = 0.31% = 0.70%

Standard Error of Difference = 0.77.

Difference between means = 0.5.

3./.....

3. DISCUSSION OF THE RELATIVE MERITS OF THE TWO METHODS.

(a) Speed.

An absolute comparison of the time spent on analysis using two completely unrelated methods is difficult to make.

The colorimetric analyses are most conveniently carried out in batches of six, each batch taking between two and two and a half hours to complete. Another forty five minutes is spent in preparation for the next batch, washing funnels and volumetric flasks and preparing solution B (with carbamate). A single analysis takes from half an hour to three quarters of an hour to complete.

In the polarographic method, the analyses are carried out continuously and, unlike the colorimetric method, can be stopped at almost any stage for short periods (which means that less time is actually wasted). Each new solution to be electrolysed is prepared as soon as a polarograph cell and a place in the thermostat becomes available. New solutions may conveniently be prepared while the previous ones are equilibrating in the thermostat, or while their polarograms are being recorded. The time taken for the analyses of six aliquots of soil solution, to the stage of recording the polarogram, is about two hours. This does not include the time spent on developing polarograms (about ten minutes for five) or the time spent in measuring wave heights).

Perhaps/.....

Perhaps a better idea of the time factor in each method could be obtained by a comparison of the total time spent on the thirty analyses for the statistical comparison:-

Three and a half days were spent on thirty eight analyses (including eight standard determinations) using the colorimetric method. Eighteen was the largest number of analyses carried out on any one day.

Two and a half days were spent on the polarographic analysis including standards. On the last day twenty four analyses were carried out.

Thus it would appear that the polarographic method has a slight advantage as regards speed. This advantage is further amplified when the time necessary for the preparation of buffer and reagent solutions for the colorimetric method is taken into account, while, if reasonable care is exercised with the cathode capillary, less redetermination of standards is required in the polarographic method.

(b) Accuracy and Reproducibility.

The colorimetric method described, is probably the only one, which, in a comparable time, will give a precision of the high order shown in the statistical treatment of its results, while comparison with the polarographic/.....

graphic method has shown that there is no error fundamental to the method. On the other hand the standard deviation of 1.26 ppm obtained with the polarographic method is reasonably good for the concentration range encountered in this sort of work and may prove to be sufficient accuracy for the diagnosis of zinc deficiency in crops.

(c) Economy.

A good polarograph may mean a considerable capital outlay, but in a laboratory already equipped with one of these instruments, the polarographic method would prove most economical. It should be borne in mind, too, that a great deal can often be accomplished with very simple equipment.

Almost every modern analytical laboratory is equipped with a spectrophotometer or a good electrophotometer so that for the colorimetric method the acquisition of the necessary equipment should present no problem.

With regard to chemicals the polarographic method should prove particularly economical but if attention is given to the recovery of the used carbon tetrachloride, the expense of the colorimetric method should not be very great.

(d) Technique.

Any polarographic method may present certain difficulties in technique for the inexperienced analyst. In the method described here, further time would have to be spent on the investigation of the effect and control of pH, especially at the stage when the fluoride is added, before it could confidently be applied to a programme of routine analysis.

The colorimetric method presents few difficulties with regard to technique.

Conclusion.

The colorimetric method was selected for the survey described in the final part of this thesis for the following reasons:-

- (i) As the colorimetric method gives more accurate results, the critical levels of zinc concentration in soils and plants could be better determined by its use.
- (ii) As the colorimetric method is - for the reasons discussed above - more likely to be used in this and other laboratories for future trace element research and as the survey represents an initial part of a much larger programme of research, it would be of advantage in the comparison of results to use the same method throughout.

PART IV./.....

P A R T IV.

THE ZINC STATUS OF CITRUS AND PINEAPPLE LEAVES AND
SOILS IN THE EASTERN CAPE.
- A PRELIMINARY SURVEY.

1. INTRODUCTION.

The object of this part of the work was two-fold.

It was proposed, by carrying out a preliminary survey, analysing samples from a wide area, to lay the foundations of a much more thorough programme of research. It was hoped to form some idea of what was to be expected from analyses in the various areas and to learn approximately, the critical levels of zinc concentration dividing the healthy plants from those suffering from zinc deficiency.

Secondly, such a preliminary survey, would test the analytical method selected under a variety of conditions and demonstrate the value of a thorough preliminary research into the analytical methods.

Citrus and pineapples are the two major crops of the Eastern Cape Province. It has been reported from the United States of America (40), that zinc deficiency in citrus is probably more widespread than any other deficiency except nitrogen, and its characteristic symptoms have been reported from every citrus-growing area in that country. In South Africa Beyers (41) and Naude (42) reported/.....

reported the widespread occurrence of zinc deficiency symptoms in the citrus areas of the Western Province and Transvaal respectively. The citrus-growing areas of the Eastern Cape are no exception and as a result of the work of Matthews (43) on zinc sulphate sprays in the Sundays River Valley, the spraying of zinc solutions on citrus trees has become a fairly common practice.

Lyman and Dean (44) reported the occurrence of zinc deficiency in the pineapples of Hawaii. Similar symptoms have been noticed in the pineapple plantations of the Eastern Cape and some farmers have adopted the practice of fertilising the plants with zinc sulphate.

Although a considerable number of leaf analyses have been carried out in the United States of America (45) and Hawaii (44), in order to determine and establish zinc deficiency levels in citrus and pineapples, similar studies have not been reported from South Africa.

Zinc deficiency commonly occurs in crops grown in countries with a large proportion of bright sunny days. In citrus, the deficiency is characterised by a typical mottling effect in the young leaves (see photograph Fig. 15), that is, the symptoms are more noticeable during the growing period when the trees are forming new leaves and shoots. Trees suffering from severe zinc deficiency show the characteristically mottled small leaves at the tips of shoots followed by multiple bud formation and die-back. Fruits tend to remain comparatively small and yield is drastically reduced. In pineapples/.....



FIG. 15.

ZINC DEFICIENCY IN CITRUS
(LEFT HAND LEAF IS NORMAL).



FIG. 16.

ZINC DEFICIENCY IN PINEAPPLES.

In pineapples there apparently exist two phases in the symptoms of zinc deficiency. The more common and less severe is a mottling and blistering of the upper surfaces of the leaves. A secondary symptom, representing severe zinc deficiency is a marked curvature of the leaves and heart of the plant (Fig. 16). Plants that exhibit this leaf curvature produce fruits or secondary growth only after considerable delay. Leaf curvature is most pronounced at the early stages of growth, the plants apparently recovering on approaching fruiting age.

2. SAMPLING PROCEDURE.

(a) Citrus Areas:

In order to obtain a fairly representative picture of the zinc status of citrus in the Eastern Cape, samples were collected from most of the areas where citrus is grown. Except in the case of orchard T (Sundays River) all the trees sampled were Navels. The areas sampled were so selected as to include high-yielding and low-yielding orchards as well of trees of different age. In each orchard sampled, ten trees of uniform growth and development were selected, from which composite leaf and soil samples were taken. The leaf sampling technique was that described by Chapman and Brown (45). The procedure/.....

cedure adopted was as follows:-

20 to 25 leaves were taken from each tree and the 200 to 250 leaves so obtained were mixed to form one sample. The leaves selected from the trees were the so-called spring cycle leaves from fruit-bearing twigs, that is, one leaf of more or less average size was selected from behind 20 to 25 fruits on each of the ten trees. Wherever possible, a similar sample consisting only of leaves showing the characteristic mottling of zinc deficiency, was taken.

A composite soil sample was obtained from around the ten selected trees by mixing sub-samples taken at points varying between 4 and 8 feet from the tree stem and situated all around the tree. The sub-samples were taken with a stainless steel sampling tool at depths varying from 2 to 20 inches.

(b) Pineapple Areas:

The same general principles were adopted when sampling the pineapple regions. As many different soil types as possible were selected in the pineapple-growing areas of the Eastern Cape. In each selected area, leaf and soil samples were taken from ten healthy plants and from ten plants which showed zinc deficiency symptoms. The plants selected were of uniform growth and similar age. Samples were only taken from the

smooth/.....

smooth Cayenne variety. Leaf samples were obtained from fully grown leaves slanting at an angle of more than 45° from the vertical. These leaves were pulled out at the base of the plant and the white, meristematic basal portion of the leaf was cut off and kept as the sample (44). Three to four leaves were sampled from each of the ten plants and mixed to give one composite sample. The composite soil sample was obtained by mixing sub-samples taken at a depth of 1 to 6 inches all around and close to the ten plants.

3. ANALYTICAL PROCEDURE.

(a) SOIL ANALYSIS.

(i) Preparation of Samples for Analysis.

The soil samples were spread out on drying paper in a room free from chemical fumes and allowed to dry for two or three days. They were then ground up with a wooden pestle in a porcelain mortar, sifted through a 1 mm. nylon mesh and quartered to give a convenient amount of sample. The quartered samples were stored in glass bottles with bakelite screw caps.

(ii) Analysis for Total Zinc.

Soil solutions for total zinc analysis were prepared from 100 mesh samples by the hydrofluoric acid - perchloric acid evaporation technique of Sherman and McHargue (46).

About a gram/.....

About a gram of the soil, prepared as described above, was finely ground in an agate mortar to pass a 100 mesh nylon screen and about half a gram of the resulting material was weighed accurately into a platinum crucible. 1 ml. water, 1 ml. of A.R. perchloric acid and 3 ml. of hydrofluoric acid were then added and the contents of the crucible evaporated to dryness on a sand bath. This process was repeated until practically all of the silica had been destroyed and driven off. A final evaporation with 1 ml. of perchloric acid alone was then carried out. The crucible was cooled and about 5 ml. water and 1 ml. of redistilled constant-boiling-point hydrochloric acid added. It was then warmed to dissolve the contents.

This solution was transferred to a separatory funnel for the analysis which was carried out by the colorimetric method described on page 51.

(iii) "Acid Soluble" Zinc.

As an approximate guide to the "available zinc" content of the soil an "acid soluble" extract, as proposed by Wear and Sommer (47), was prepared by the following method:-

10 grams of the soil sample (1 mm. mesh) were weighed into a wide necked 100 ml. conical flask and 50 ml. of tenth normal hydrochloric acid (N = .1000 - prepared from redistilled constant-boiling-point acid) added.

The mixture was/.....

The mixture was then shaken intermittantly for three hours and filtered through a Whatman No. 40 filter. (This remained dry until filtering and the residue was not washed). A suitable aliquot (10 ml. for most soils, 20 ml. for zinc deficient soils) was then pipetted into the separatory funnel for the analysis.

(b) LEAF ANALYSIS.

(i) Preparation of the Samples for Analysis.

Citrus:

The leaves were washed, one by one, in distilled water using cotton wool swabs. They were then dried between sheets of drying paper and the centre ribs cut out with stainless steel scissors. The leaves were then placed in muslin bags, dried for about twelve hours at 70 - 80°C in a forced draught oven. The dried samples were then ground to a fine powder in a mechanical agate ball-mill and stored in wide-mouthed specimen tubes (cork stoppers). Before the analysis the powder was dried at 105° overnight.

Pineapples:

The white basal portions of the leaves were further trimmed of any green leaf material and washed as for citrus leaves. The leaf portions were then cut into fine pieces across the fibres, a precaution which facilitated the grinding./.....

grinding.

The procedure after this stage was exactly as for the citrus samples.

(ii) Digestion of Leaf Samples.

It has been found that the normal dry-ashing procedures are unreliable unless carried out with extreme care. Moreover, appreciable quantities of the trace elements are entrained by the silica. The following wet-digestion procedure, developed by the Soil Science Section of the Rhodes University Chemistry Department, was therefore adopted.

Apparatus:

- (a) A hotplate with a good heat control system.
- (b) A set of 12 to 24 digestion flasks with reflux covers. The design of the flasks used, which were made in the Chemistry Department, is shown in Figure 17. A is a wide-necked 150 ml. erlenmeyer flask, made from an ordinary erlenmeyer flask by widening the neck. Two small indentations (E), made in the lip of the flask, allow the escape of excess fumes. B is the reflux cover, consisting of an inverted, funnel-shaped cone fitting into the neck of A, a short glass rod C fused into the funnel for lifting B, and a short glass rod D, with a tapering point fused on to the apex of B. Vapour from the boiling acid in A condenses on the lower surface of B and flows back along D into the liquid. The constant drip-ping-back of liquid in the centre reduces the danger of bumping and promotes even and gentle boiling. As the cover acts as a reflux condensor a big saving in nitric acid is obtained.

Reagents:/.....

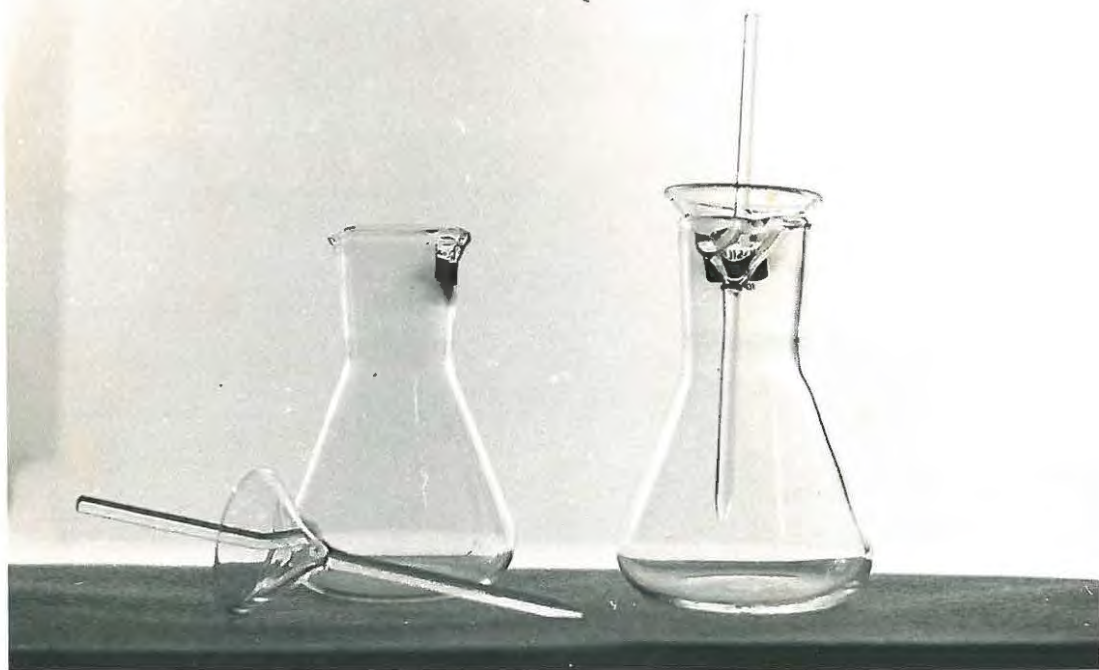
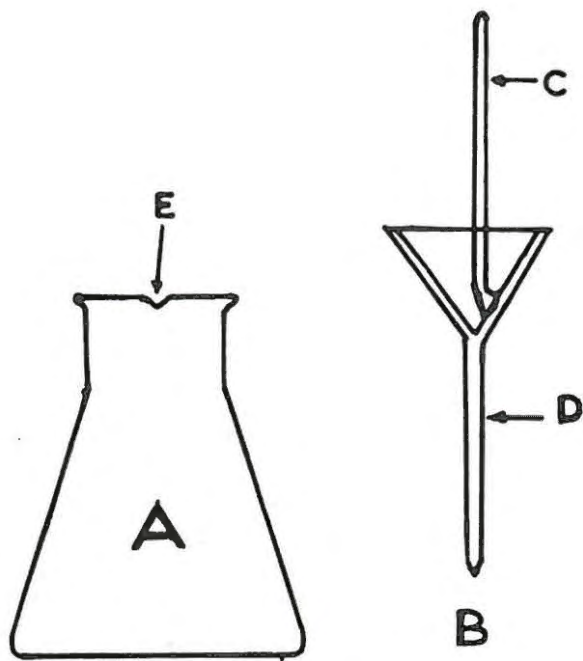


FIG. 17.
DIGESTION FLASK.

Reagents:

(a) Nitric Acid:

It was found that ordinary C.P. nitric acid contained a negligible amount of zinc.

(If a blank determination carried out on the acid shows the presence of an appreciable amount of zinc, the acid should be redistilled from an all-pyrex-glass still).

(b) Perchloric Acid: A.R. quality 60-70% acid was used.

Procedure:

About half a gram of the dried plant material was weighed into a digestion flask and 10 ml. of C.P. nitric acid added. The reflux cover was then placed on the flask and its contents digested at low temperature on the hot plate until the initial vigorous reaction had subsided. (Care must be taken at this stage to avoid the contents of the flask frothing over). The acid mixture was then boiled gently until most of the material had gone into solution. 2 ml. of perchloric acid were then added and the digestion continued at a gentle boiling rate for half an hour to an hour until the solution was a pale yellow colour. The cover-glass was then removed, the underside rinsed with a few ml. of zinc-free water, and the contents of the flask evaporated to just moist with the hot plate at a slightly higher temperature. The residue was taken up in 10 ml. of zinc-free water and
this/.....

this solution filtered hot through a Whatman No. 40 filter, washing with small volumes of hot water. The filtrate was evaporated to a convenient volume (about 20 ml.) and analysed for zinc by the colorimetric method (page 51), the whole of the solution being used.

The digestion, as described above, was found to proceed with remarkable smoothness. Tests carried out in the Soil Science Section of the Chemistry Department have shown it to be very accurate, safe and reproducible. It eliminates the introduction of large quantities of sulphuric acid, which takes place in the normal nitric - perchloric - sulphuric acid wet-digestion procedure. It was found that 5 ml. of nitric acid were sufficient for the digestion of 0.5 g. of material, but that, with 10 ml., the process occurred more smoothly. 10 ml. of nitric acid were found to be sufficient for the digestion of up to 2 g. of leaf material.

It was found that 24 leaf solutions could comfortably be prepared in one day by this method.

3. RESULTS./.....

3.

RESULTS.

(a) ZINC IN CITRUS.

The zinc contents of the citrus leaves and soils analysed are shown in Table X below.

TABLE X.

No.	AREA.	TREATMENTS.	Zn-DEFICIENCY		SOILS.		LEAVES.	
			SYMPTOMS.	pH.	Total Zn ppm.	Acid Sol. Zn.	Zn in Normal	Zn in Mottled.
1	Kat River.	N.	Pronounced.	8.0	40.0	1.8	16.4	10.9
2	"	N.	Severe.	7.6	42.2	2.0	13.0	6.6
3	"	N,P,K (1946-54). Zn Sprays ('51 - '54).	Absent.	6.6	42.3	8.2	48.2	
4	"	Controls Zn Sprays ('51 - '54).	Absent.	8.3	43.2	5.1	28.1	
5	"	N+Zn (1951 - 1954).	Absent.	8.5	44.2	7.4	49.0	
6	Belmont Valley.	N,P,K (1946-54).	Slight.	5.5	33.4	4.8	14.1	
7	"	Controls.	Pronounced.	6.2	35.2	4.5	11.3	
8	"	N.	Severe.	7.8	25.3	0.9	14.6	
9	"	N.	Pronounced.	7.9	26.6	2.2	10.8	
10	Fish River.	N.	Slight.	8.6	72.8	3.4	15.1	
11	"	N.	Severe Fe Chlorosis.	8.9	71.1	2.5	17.2	
12	Sundays River.	N.	Severe.	8.2	42.7	6.5	10.0	8.9
13	"	N + Zn Sprays.	None.	8.9	70.0	8.0	56.0	
14	"	N + Mn + Zn Sprays.	Severe.	8.1	75.0	4.7	11.7	8.0
15	"	N + Cu + Zn Sprays.	Severe.	8.4	62.1	8.4	5.9	4.0

NOTE: /.....

NOTE: Samples 12 - 15 were obtained through the co-operation of Mr. I. Matthews, Officer in Charge, Addo Experimental Station. All the other samples were obtained through the co-operation of Mr. C.A. Lombard, Field Officer of the Citrus Exchange.

All of the samples except 13 are from navel orange trees.

(b) DISCUSSION OF RESULTS FOR CITRUS.

Most of the orchards sampled received nitrogen treatments only (in the form of ammonium sulphate). Orchards 3 and 4 and 6 and 7 represent N,P,K trials carried out by the Citrus Exchange since 1946. Some of the orchards sampled received minor element sprays as indicated. All the orchards were sampled during November, 1954, approximately six months after the application of the zinc sprays.

(i) Zinc in the Leaves.

According to Chapman and Brown (45), zinc deficiency symptoms are noticed in citrus orchards in the U.S.A. when the zinc content in the three-to-seven month old Spring-cycle leaves drops below 15 ppm. High yielding trees have an average zinc content of 30 ppm in the same leaves. It would appear, from a comparison of the zinc contents found and the corresponding symptoms shown by the leaves, that the/.....

that the critical level of zinc concentration at which zinc deficiency symptoms begin to appear is slightly higher in the Eastern Cape orchards than that reported by Chapman and Brown for American orchards. For example, in orchard 1, where 16.4 ppm zinc was found in the leaves, zinc deficiency symptoms were fairly pronounced, while in orchard 4, with 28.1 ppm in the leaves, deficiency symptoms were absent.

It was tentatively suggested that, when the zinc content in the leaves falls below 20 ppm, deficiency symptoms may be expected to occur in citrus in the Eastern Cape.

On the ^{is} basis most of the orchards sampled must be regarded as suffering from zinc deficiency. The exceptions are orchards 3, 4, 5 and 13 which have received annual sprays of zinc sulphate. These orchards may be regarded as healthy, on American standards, a fact which lends support to the recommendation of Matthews (43) that zinc should be sprayed regularly. Orchard 13, with the highest zinc content, was also the highest yielder of all the orchards sampled.

The fact that orchards sampled in the Sundays River Valley, 14 and 15, show severe zinc deficiency, in spite of having been sprayed with zinc, presents a problem which cannot be answered with any finality at this stage. However, certain suggestions can be made when one compares the treatments more carefully. Sample 12 can be taken as the control; it received only nitrogen, and

the zinc/.....

the zinc in the leaves was 10.0 ppm with severe deficiency symptoms. Orchard 13 received nitrogen (ammonium sulphate) and a zinc spray, with a consequent "increase" of the zinc content of the leaves to 56 ppm. 14 received nitrogen and a spray containing a mixture of manganese and zinc, with no subsequent increase in the leaf zinc (12 ppm.). 15 received nitrogen and a spray containing a mixture of copper and zinc. The extremely low zinc content of 5.9 ppm in the leaves suggests that a suppression of the normal uptake of zinc from the soil actually occurred. The evidence therefore points to an interaction between zinc and the metals manganese and copper in particular, when these are applied as mixtures and it appears that in such cases the trees are not utilising the applied zinc. A similar antagonism between zinc and copper was found in the case of the pineapple analyses.

(ii) Zinc in the Soils.

The total zinc content of all the soils sampled was found to be low, except those in the Fish and Sundays River areas. (In the Sundays River samples the high apparent zinc content may be due to drippings from the leaves during spraying, as in 12, an unsprayed orchard, the total soil zinc was only 42.7 ppm.). In most areas, therefore, zinc deficiency is an inherent problem, rather than one of availability, though the high pH of some of the soils undoubtedly depresses the availability of what
little/.....

little zinc there is. Soils with a low pH tended to have a smaller total zinc content than more alkaline soils. This is probably due to the fact that under more acid conditions this zinc is more soluble, more easily leached and more quickly used up by the perennial trees. (Most of the trees were more than 20 years old).

There is no apparent relationship between the "acid soluble zinc" values and zinc deficiency symptoms.

(iii) Effect of Treatment on Zinc Uptake.

The most outstanding examples of the results of treatments on zinc uptake were samples 3 and 4. The trees of 3 have received annual applications of nitrogen, phosphorus and potassium since 1946 and zinc sprays in every winter since 1951. The trees represented by sample 4 are in a control plot in the same orchard. These trees have had the same zinc sprays but no treatment with nitrogen, phosphorus and potassium. The zinc content of 3 is very much greater than that of 4, despite the fact that the total zinc content of the soil is about the same for both samples. It is suggested that this phenomenon can be attributed to the fact that the continuous application of ammonium sulphate to sample 3 has brought down the pH of the soil by nearly two units (8.3 to 6.6), thus increasing the availability of the soil zinc and increasing the uptake of zinc by the trees. It is interesting to note the increase in acid soluble zinc at the lower pH.

A similar/.....

A similar small increase in the uptake of zinc was shown by samples 6 and 7, but here the trees in the control plot (7) were in very poor condition and there was obviously something seriously wrong with their growth. In this case too the soil was apparently acid before treatment, so that the change was from slightly acid to more acid rather than from alkaline to acid as in the case of 4 and 3.

It would appear that, where the soil is alkaline and its total zinc content high (e.g. in the Fish River area), a considerable improvement in zinc uptake could be obtained by bringing down the pH of the soil with good nitrogen treatments with ammonium sulphate. (Analyses have shown that most orchards in the Eastern Cape are deficient in nitrogen when compared with American standards).

(c) ZINC etc./.....

(c) ZINC IN PINEAPPLES (CAYENNES).

The zinc contents of the pineapple leaves and soils analysed are shown in Table XI below.

TABLE XI.

No.	AREA.	TREATMENT.	AGE AND PLANTING MATERIAL.	SOILS.		LEAVES.	
				pH.	Total Zn ppm.	Acid Sol. Zn ppm.	Zn ppm.
1(a)	Bathurst.	N.	14 months, tops.	4.8	29.5	0.2	31.3
1(b)	"	N + Zn Spray.	" "	4.9	42.2	0.2	27.4
2(a)	Bathurst.	None.	12 mths., suckers.	4.9	30.0	0.2	25.5
2(b)	"	N+Cu+Zn mixt.	" "	4.9	51.0	7.3	21.9
3(a)	Kidds Beach,	None.	22 months, tops.	4.5	25.2	0.5	35.9
3(b)	"	N+Cu+Zn mixt.	" "	4.1	64.6	1.6	21.9
4	Kidds Beach.	N.	6 months, slips.	5.6	106	0.9	32.8
5	"	None.	" "	5.4	70	8.4	21.2
6(a)	Kei Mouth.	None.	13 mths., suckers.	5.6	52.1	0.7	18.5
6(b)	"	N+Cu+Zn mixt.	" "	5.3	65.6	1.7	24.1

(d) DISCUSSION etc./.....

(d) DISCUSSION OF THE RESULTS FOR PINEAPPLES.

All the samples were taken during March and April, 1955. The plants received no other treatments than those recorded. All the plants showed severe zinc deficiency symptoms when young, but apart from 2(a) and 2(b), which showed slight symptoms, none of the pineapples showed any signs of zinc deficiency when sampled.

(i) Zinc in the Leaves.

According to Lyman and Dean (44) zinc deficiency symptoms appeared in the pineapples of Hawaii when the zinc content of the white basal leaf tissue of the plants dropped below 20 ppm. Healthy plants contained an average of 44 ppm. On this basis the figures reported in Table II would indicate that most of the plants sampled lie between deficient and healthy. It does seem likely, though, that the critical level of zinc content is slightly higher than reported from Hawaii.

It was noticed that plants, which had shown the symptoms of zinc deficiency very strongly when young, seemed to have recovered completely as they grew older, irrespective of whether they had received zinc treatment or not.

The following extract from Table XI makes this clear:-

<u>Sample.</u>	<u>Age.</u>	<u>Zinc Content.</u>
5	6 months.	21.2 ppm.
2(a)	12 "	25.5 "
1(a)	14 months.	31.3 "
3(a)	22 "	35.9 "
		These/.....

These results are, perhaps, not strictly comparable, as the samples, except 1(a) and 2(a), do not come from the same areas. Except in the case of 5, however, there is no great difference in pH or total zinc content of the soil. The youngest, 5, although grown in soil of high total zinc content, nevertheless has the lowest zinc content in the leaves of this group. 3(a), the oldest, on the other hand, has attained an almost healthy zinc content in the leaves even though the total zinc in the soil is low.

The plants from which these four samples were taken received no zinc treatment and were grown on virgin soils.

Thus it would appear that the plants are able to obtain sufficient zinc from the soil once their root systems are better developed, even when the total zinc content of the soil is low.

(ii) Zinc in the Soil.

All the untreated soils, except the heavy doleritic types around East London (4,5,6) were found to have a low total zinc content and a very low acid-soluble zinc content. As was the case with the citrus, soils with higher pH values had higher zinc contents than the more acid soils, which were inherently deficient in zinc. As was to be expected, treatment with zinc raised both the total and the acid-soluble content of the soil.

(iii) Effect/.....

(iii) Effect of Treatment on Zinc Uptake.

When the results were first examined a number of anomalies became immediately apparent. Instead of the substantial increase in the zinc content of the leaves to be expected after treatment with zinc, the plants showed either a very slight increase in zinc content (6b), or more commonly a definite depression of the zinc content.

These results will probably be best understood if something is first said about the fertilisation of pineapples. It has become almost standard practice for pineapple farmers in the Eastern Cape to apply the so-called "pineapple mixture" to young plants a few months after planting. This mixture consists of 4 lbs. of zinc sulphate plus 4 lbs. of copper sulphate made up to 100 lbs. with ammonium sulphate. This mixture is applied to the soil around the plants at the rate of 25 lbs. per 1000 plants. This treatment may be repeated several times during the life of the plants.

1(a) and 1(b) are closely comparable samples, the plants being in the same field, of the same age, and grown from the same planting material (tops). Though these plants showed severe symptoms of zinc deficiency when young, they showed none at the time of sampling and there was no difference in the size or health of the plants. Sample 1(b), in addition to ammonium sulphate, had received a zinc sulphate spray, in which the zinc had been precipitated with sodium hydroxide.

The results/.....

The results show that the zinc spray increased the zinc content of the soil, while the zinc content of the leaves seems to have been slightly reduced. This effect may be due to the use of sodium hydroxide instead of the usual slaked lime to neutralise the acid residual effect of zinc sulphate, the localised, strongly alkaline conditions influencing the uptake of zinc adversely.

2(a) and 2(b) were samples of plants of the same age grown from suckers on the same land. 2(b) received two treatments with the so-called "pineapple mixture". The lower zinc content of the leaves of 2(b) is significant, especially in view of the substantial increase in the zinc content of the soil.

3(a) and 3(b) were both grown from tops planted at the same time on virgin soil. 3(b) also received two treatments with "pineapple mixture" and, while the soil shows a greater increase in zinc content than in the case of 2(a) and 2(b), there is a greater drop in the zinc content of the leaves.

Samples 6(a) and 6(b) were from plants of the same age, grown from suckers on virgin land. 6(b) received one application of "pineapple mixture". Here the plants showed a slight increase in zinc content.

Although/.....

Although a long period of investigation would be necessary before the problem of zinc treatment for pineapples could be solved, the results do seem to indicate that there is some interference with the uptake of zinc in the case of the treated plants. Certainly the "pineapple mixture" is unbalanced, as a healthy plant normally requires only one fifth as much copper as zinc, and it seems likely that copper enters into competition with the zinc in being taken up by the plant. Other factors too, such as pH, undoubtedly play a part.

5. CONCLUSION.

Although this brief survey does not provide the material for any certain conclusions with regard to citrus or pineapples in the Eastern Cape, its results are of sufficient interest to show the extreme importance of carrying out soil and leaf analyses in collaboration with experimental work in the field.

As a result of this work a comprehensive experimental programme has been undertaken at Rhodes University to study the apparent antagonism between zinc and copper more closely. This study will involve the complete life cycle of the plant and will take many years to complete.

S U M M A R Y.

For this thesis a critical study has been made of the colorimetric and polarographic methods for the determination of zinc in soils and plant materials. The work is divided into four parts.

Part I deals with the colorimetric methods. A critical review of the published colorimetric methods is made, with the reasons for selecting the method of Cowling and Miller for a full practical study.

An account of experimental investigation of various aspects of the method follows. These include the preparation of standard zinc solutions, the storage of solutions, the transmission curves of carbon tetrachloride solutions of dithizone and its zinc complex, the influence of pH on the extractions, pipetting and extracting techniques and the stability of dithizone and dithizonate solutions.

As a result of these investigations the original Cowling and Miller method was modified and a full description of the modified method is given.

To test the precision of the modified method, thirty analyses were carried out on the same solution and the results examined statistically. Photometer measurements for each of the analyses were made with a Cenco Sheard "Spectrophotometer" at 520 m μ and 535 m μ as well as with a Hilger "Spekker Absorptionmeter" using a green filter. The relative merits of the three methods of photometry are discussed. A description/.....

A description of the investigation of the possibilities of a carbon tetrachloride - di-beta-naphthyl-thiocarbazonium method follows. The investigations of various practical considerations, such as pH and reagent concentrations are described and conclusions drawn. The method was shown to be poorly reproducible due to rapid deterioration of the di-beta-naphthyl-thiocarbazonium solution. For this reason the method was discarded.

Part II deals with the polarographic methods.

A short review of the published methods is given, together with a more detailed description of the method of Reed and Cummings, which was at first selected for study. A short criticism follows.

The apparatus used is described.

An account is then given of the experimental steps which led to the development of a new polarographic method, in which interfering ions are complexed with fluoride and thiocyanate. This method dispenses with all preliminary separations. A full description of the new method is given.

An investigation of the effect of the presence of other ions is described and the potentialities of the method are discussed.

In Part III/.....

In Part III an account is given of a statistical comparison of the colorimetric method and polarographic method. Standard deviations of 1.67% and 4.0%, respectively, were obtained, while no significant difference in the results given by the two methods was found. The relative merits of the two methods are discussed.

Part IV contains a brief survey of the zinc status of citrus and pineapples in the Eastern Cape. Sampling and analytical procedures are described and the results are discussed.

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