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# MICRONUTRIENTS IN THE NUTRITION OF COCONUT

## I. METHODS AND PRELIMINARY INVESTIGATION

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## SUMMARY

As a prelude to a comprehensive investigation on micronutrient requirements of coconut palms, preliminary studies on sampling procedures, choice of plant materials and analytical methods have been carried out.

Toddy as a plant material was found to be unsatisfactory for nutritional studies on micronutrients because of the inconsistent diurnal and seasonal fluctuations, and the wide variations between palms.

Among leaf materials the younger leaves for copper and boron and older (probably 14th) leaves for iron and manganese have shown promise of providing information on their status in palms.

## INTRODUCTION

The turn of the present century saw the rapid increase in a list of elements proved to be essential for normal growth of plants, but required in amounts less than 1.0 ppm in culture media. Among these at least six elements, namely, iron, manganese, copper, zinc, boron and molybdenum were demonstrated to be universally indispensable for normal plant growth, while others such as sodium, chlorine, aluminium, vanadium and several others were shown to promote better growth in certain species of plants. Various terms have been used to describe the former group of essential plant nutrients, but in this series of papers the more appropriate term micronutrient will be used for reasons adduced by Arnon (1950).

### Investigations on coconut

The short discussion that follow is meant only to highlight some of the interesting findings, and should not be considered to represent a comprehensive review of literature.

Some of the earliest references to investigations on micronutrients in coconut palms were in relation to diseases such as the "Unknown" disease of Jamaica (Innes, 1950), the "Root-wilt" disease of India (Sankarasubramony *et al.*, 1951) and the "Yellowing" disease of Sri Lanka (Nethsinghe, 1959). In these the scope of study was generally restricted to a simple comparision of the micronutrient contents of leaves from healthy and diseased plants. Davis and Pillai (1966) however, reported the results of a more comprehensive investigation on the effects of micronutrients on the "Root-wilt" affected palms. They found that application of micronutrients brought about a general increase in nut yield of both affected and healthy palms, but they were unable to relate the cause of the disease to a micronutrient deficiency.

The influence on germination of micronutrients injected into the husk of seednuts was investigated by Sumathykutty Amma (1964). It was found that application of micronutrients generally produced a marked increase in the rate of germination. The effect of boron was of particular significance as it reduced the period of germination by about a third compared to controls. It may be implied from this that though mother palms may not display any symptoms of deficiency, their seednuts could be at sub-optimal levels with respect to reserves of micronutrients.

During the past decade considerable work has also been carried out in the South Pacific Islands by Fremond (1961), Meadows (1964), Pomier (1964, 1967) and by Southern and Dick (1968). In most of these islands the calcareous nature of the soil combined with the low levels of organic matter posed unique problems on nutrition. The development of simple procedures to overcome deficiencies occurring under these conditions, and the evaluation of provisional lower limits of sufficiency for micronutrients in the 14th frond of adult palms, are some of the features of these investigations.

## Work reported in Sri Lanka and the present position

In Sri Lanka the investigations into the problem of magnesium deficiency of coconut palms in the heavily leached lateritic soils of the South and South-Western regions indicated that under such conditions, the leaves of affected palms could have contents of iron and manganese significantly lower than those of healthy plants. However, this was considered to be a secondary effect caused by a deficiency of magnesium (Nethsinghe, 1959). Likewise the investigations on the "Leaf-scorch" disease of coconut at one stage appeared to be associated with a deficiency of boron, as was evident by the short-lived recovery of plants to sustained foliar sprays of boron solutions, and by the presence of lower levels of boron in the fronds of diseased palms (Nethsinghe, 1964).

Paltridge and Santhirasegaram (1957), Santhirasegaram *et al.* (1965) and Santhirasegaram (1967) studied the nutrient status of some typical coconuts soils of Sri Lanka by the bio-assay technique. Using species of grasses and legumes as indicator plants, they found that legumes alone responded significantly to applications of boron on the poor lateritic soils of the South and South Western Provinces of Sri Lanka. Similar observations were made by them on the white silica sands (commonly known as cinnamon sands) of the North-Western Province. However, it has not been possible to assess the significance of these observations because of the scant information available on the requirements of these nutrients for coconut palms.

It is relevant that sporadic occurrences of acute deficiencies of micronutrients have been reported in other plantation crops in Sri Lanka. For instance Tolhurst (1954, 1963) found two rare and isolated cases of the occurrence of manganese deficiency in tea plants, caused by a localised change in the soil reaction. Tolhurst (1962) also diagnosed and treated successfully incidence of zinc deficiency in tea plants. In rubber plantations, Jeevaratnam (1958) detected two isolated cases of zinc deficiency in Galle District and at Monaragala. Investigations however, suggested that this was caused by the non-availability of zinc as a result of some soil reaction, and not due to a general deficiency of it in the soil.

The information available does not provide any useful guidance to the micronutrient status of the coconut soils of Sri Lanka. For this reason it was decided to carry out a comprehensive study to evaluate (*a*) the requirements of essential micronutrients for the healthy growth of coconut palms and (*b*) the micronutrient status of the typical coconut soils of Sri Lanka.

The main object of this paper is to introduce some of the techniques that are to be used during the course of the investigations and also to present some preliminary information on sampling procedures for plant materials. Except where modifications are introduced, discussion on details of these techniques and procedures will be omitted from the subsequent papers.

## EXPERIMENTAL

### (a) Leaf sampling procedure

It is generally recognised that the leaf is the most suitable material to assess the nutritional status of plants. However, apart from nutrient supply or availability, several other factors can affect the mineral composition of the leaf. Hence for the correct interpretation of leaf analytical data, initially a proper sampling procedure must be established taking into consideration the influence of maturity and region of leaf, and the season and time of sampling, on the mineral composition of leaves.

Ziller and Prevot (1963) considered the 14th frond from apex to be the most satisfactory for investigations on macronutrient status of adult palms, while work at the Coconut Research Institute of Sri Lanka have shown that the 1st and 6th leaf from apex to be more satisfactory than mature fronds for investigations on magnesium deficiency (Nethsinghe, 1962). It has also been observed that diurnal variations and gradients in the mineral composition occur both within fronds and within leaflets in coconut palms. In order to eliminate complications arising from such influences, the following general procedure for the preparation of leaf samples was adopted.

The sampling of leaves was done between 7.00 and 11.00 a.m. on a sunny day, and the frond to be sampled was initially cut down and 20 leaflets were taken from each side of the middle region. After separating the midribs (ekels) from laminae, 20 cm lengths were cut off from the distal and proximal ends of the laminae.The middle portions were washed briskly in 0.1 *N* solution of HCl containing 1.0 percent teepol and rinsed thrice in deionosed water. The samples were then dried overnight in an oven at 90° C and either hand crushed or powdered in a stainless steel micro hammer mill.

### (b) Effect of leaf maturity on micronutrient content

To investigate the influence of leaf maturity on the distribution of micronutrients, leaf samples were taken from six localities in the North Western Province of Sri Lanka. The soils in the selected areas were mostly acidic, but represented a range typical of the greater part of the coconut growing regions of Sri Lanka.

Leaf samples of the 1st, 6th, 10th and 14th fronds were prepared by combining material from 10 healthy palms in each selected plot. In assigning the leaf position the youngest fully opened leaf was considered leaf number one and the others followed in the increasing order of maturity. The samples were analysed for iron, manganese, copper, zinc and boron according to procedures described in appendices I and II.

### (c) Disc samples versus whole leaf samples

Studies were initiated to examine the possibility of using disc samples instead of whole leaf samples for nutritional studies on seedlings grown in sand cultures.

For this investigation materials were obtained from 18 month old plants treated with the full complement of essential nutrients in sand culture. The 1st and the 6th leaf from three plants were sampled by taking 10 leaflets from either side in each frond. In each leaflet the midrib (ekel) was removed and 15.0 cm lengths were cut off from either end. The samples were washed as described earlier and dried between filter paper. In place of discs V—shaped pieces were cut off from the laminae (see Fig. 1) and bulked for analysis. The rest of the material was combined to form the whole leaf sample. Each sample was then dried and hand crushed before sub sampling for chemical analysis. Fe and Mn were determined in the samples according to procedures described in Appendix I.

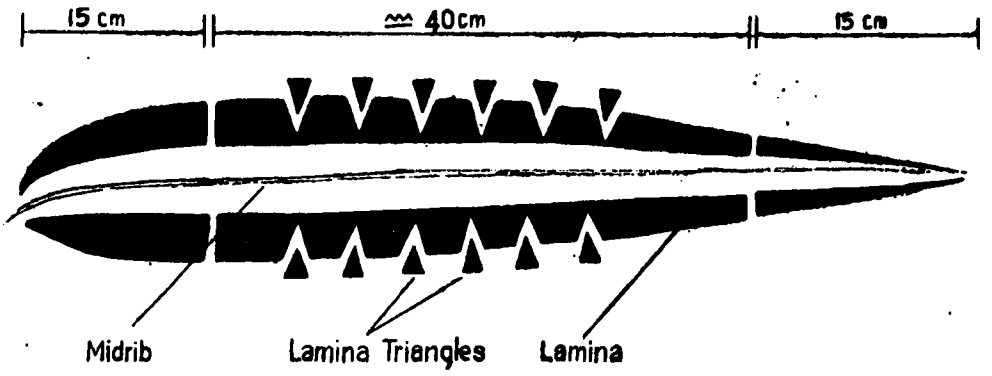



Fig. 1. Diagramatic representation of the sampling procedure for lamina "triangles."

### (d) Other plant materials

Salgado (1948) and Salgado *et al.* (1956) have shown that potassium and phosphorus contents of the liquid endosperm (coconut water or coconut milk) of ripe coconuts, reflected the nutrient status of palms. A unique finding in their studies was that the fresh liquid endosperm could be analysed directly for potassium and phosphorus without a preliminary digesting or ashing process. The possibility of using coconut water for the direct determination of micronutrients is now under investigation.

Nathanael (1955) studied the mineral composition of the sap (toddy) tapped from the coconut inflorescence and found that the contents of N, P, Ca and Mg of different palms to be remarkably constant. The potassium content however varied in the different plants. It was thought likely that the concentration of these nutrients in sap would reflect the requirements of developing inflorescence components.

In order to evaluate the usefulness of toddy for nutritional studies on micronutrients, it was decided to investigate initially the variability between palms, and also the diurnal and seasonal effects on sap composition.

Tapping of toddy on six palms commenced on 30th September, 1970 and was continued daily over a period of six months, covering a wet season (October–November) and a dry season (February–March). Every seventh day, the sap was collected in polythene lined clay pots for micronutrient analysis. Toddy was also collected at 8 hourly intervals every twenty-eighth day in similar pots for chemical examination.

## RESULTS AND DISCUSSION

### (1) Effect of leaf maturity on micronutrient content

The data presented in Fig. 2 illustrate the distribution of iron, manganese, copper, zinc and boron in leaves of different maturity in adult coconut palms. The trends are generally similar to those observed by Southern and Dick (1968) with young palms in New Guinea and Papua. A comparative study with data obtained by Southern and Dick should therefore provide a clearer picture of the leaf position that should be chosen to study the micronutrient status of coconut palms.

### (a) Iron

The Fe contents ranged from about 20 ppm in young leaves to about 40 ppm in mature leaves. In the Binguiya plantation the Fe content in the 14th frond rose to 90 ppm. It is not certain whether this was due to a higher availability of iron in this area, where deposits of iron ore are known to occur. However, it is remarkable that in spite of the acidic soil conditions Fe levels have remained relatively low in the leaves of coconut palms. The evidence basically supports the views expressed by Southern and Dick (1968), that Fe contents of mature leaves (where accumulation is likely to occur), give a better index of the iron status of palms.

### (b) Manganese

The manganese contents ranged from about 75 ppm in the 1st leaf to about 175 ppm in the 14th frond. As was expected the Mn levels in leaves of palms growing in the less acidic soils of Gallewela were consistently low. As observed by Southern and Dick (1968), there was an accumulation of Mn in mature leaves, and the wider difference in these leaves for different localities seems to reflect availability trends. As for iron therefore, the mature 14th frond could be used to determine the manganese status of palms.

### (c) Copper

Work on young palms in Papua and New Guinea have shown that the leaf Cu content decreases with age of fronds. A similar pattern is seen in the work carried out here on adult palms with respect to five of the selected sites. However, the result from Bandirippuwa Estate did not show this expected trend.

The copper contents in the youngest leaf for adult palms ranged between 4.0 and 9.0 ppm as against 8.0 and 11.0 ppm for young palms in New Guinea and Papua. The lower levels coupled with the wider differences in Cu contents for the 1st leaf in the six localities of Sri Lanka, seems to indicate that the distribution of Cu in this frond reflected its status in adult palms. It is therefore suggested that for studies on Cu nutrition, the youngest fully opened leaf be sampled according to the procedure described earlier.

### (d) Zinc

Zinc contents varied from 12–20 ppm in young leaves to about 10–22 ppm in mature leaves. Southern and Dick (1968) suggested the sampling of mature leaves for zinc studies on account of the observed differences between sites and the greater accumulation of zinc in these leaves. The work reported here however, does not show any consistent trend and hence no particular leaf position could be suggested.

### (e) Boron

The boron contents ranged from 15–38 ppm in the first leaf to 12–17 ppm in the 14th frond, and except for one odd value a consistent trend is apparent, in which the boron content diminishes and levels off with age of frond. The wide differences observed for boron contents in the first leaf indicate that this leaf may reflect the boron status of adult coconut palms.

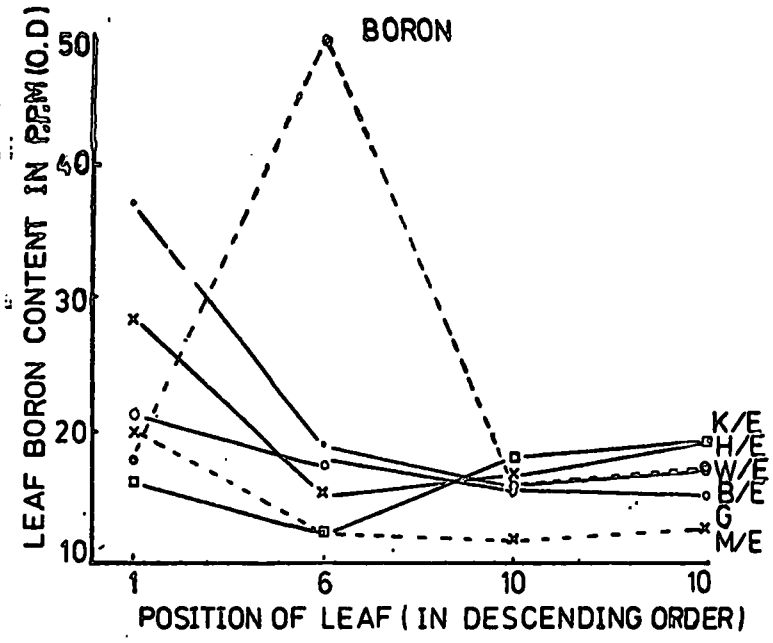
It appears therefore, that among leaf materials the younger leaves for copper and boron, and older (probably 14th) leaves for iron and manganese, may provide information on the status of these nutrients in adult coconut palms.

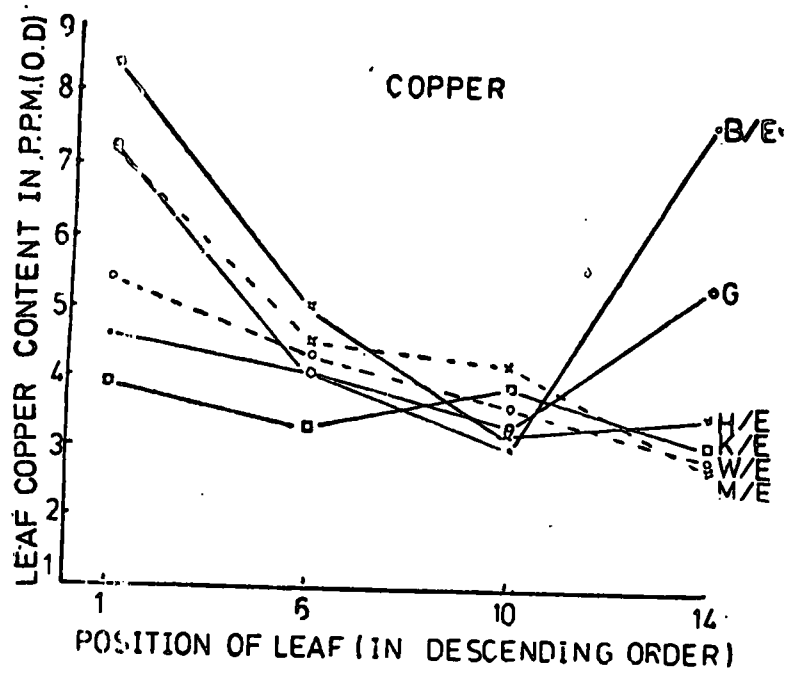
### (ii) Lamina triangles

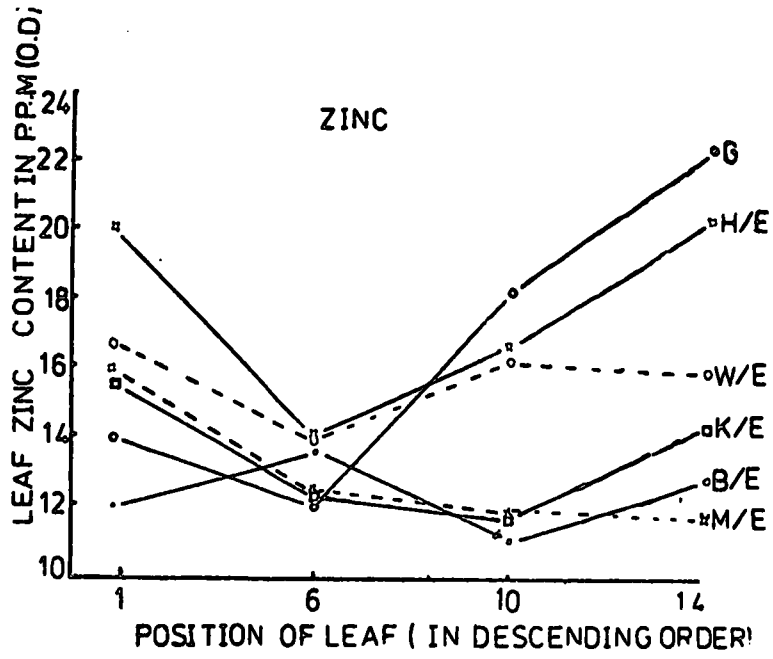
The data presented in Table 1 compares the contents of iron and manganese in "lamina triangles" with those of the corresponding samples of whole leaf.

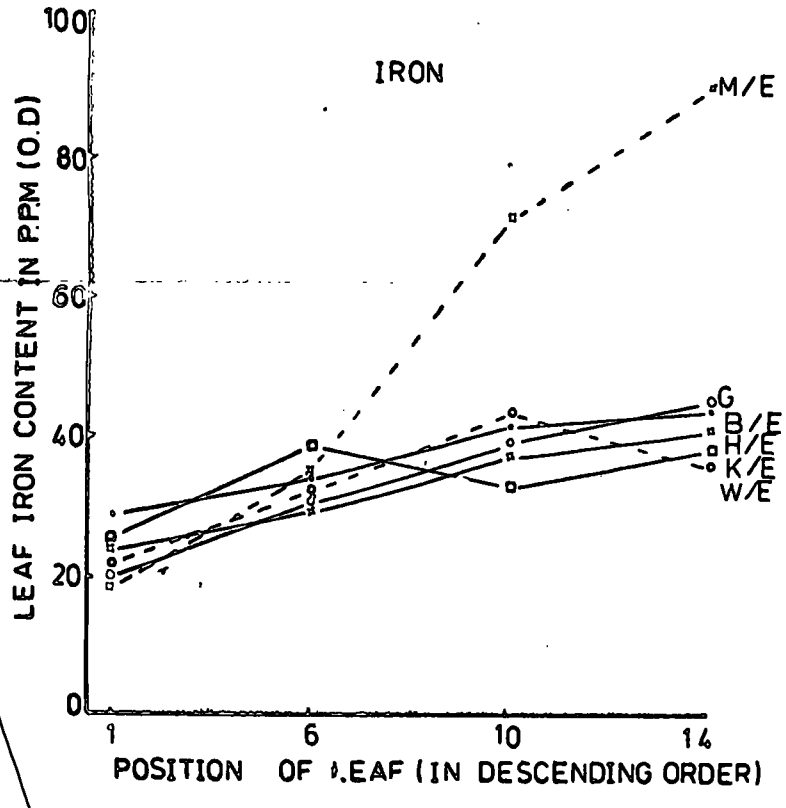
CONTENTS OF Fe, Mn, Cu, AND B, IN COCONUT LEAF SAMPLES WITH RESPECT TO LEAF POSITION

| LOCATION | SOIL TYPE | SOIL pH |
|---|---|---|
| BANDIRIPPUWA ESTATE LUNUWILA | SANDY LOAM | 5·60 |
| HOREKELLE ESTATE KUDAWEWA | CINNAMONSAND | 5·79 |
| BELIGAMA ESTATE GALEWELA | LIMESTONE DERIVED BROWN SOILS | 6·08 |
| KIRIMETIYANA ESTATE LUNUWILA | CLAY LOAM | 5·28 |
| MARANDAWILA ESTATE BINGIRIYA | SANDY LOAM | 5 70 |
| WALAHAPITIYA ESTATE NATTANDIYA | LATERITIC LOAM (GRAVEL) | 5 50 |



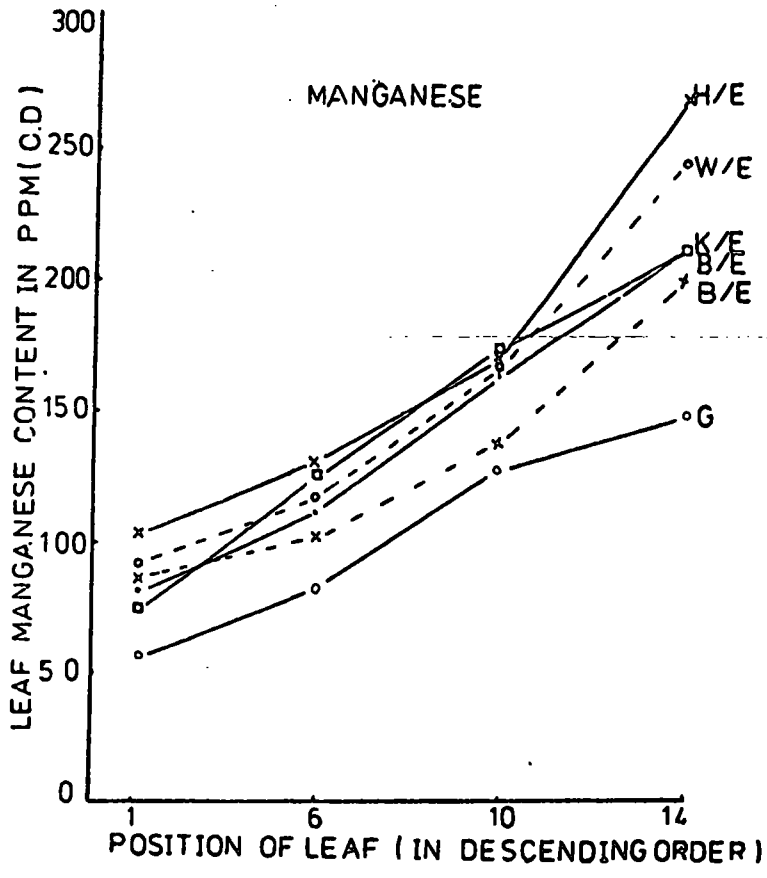




Fig. 2. Contents of Fe, Mn, Cu, Zn and B in coconut leaf samples with respect to leaf position.

**TABLE 1**

**Iron and manganese contents of leaflets compared with "lamina triangles".**

| SAMPLE | | | IRON ug Fe/gram of leaf material | | MANGANESE ug Mn/gram of leaf material | |
|---|---|---|---|---|---|---|
| | | | *Leaflets* | *"Triangles"* | *Leaflets* | *"Triangles"* |
| Plant 1, | Leaf 1 | .. | 50.00 | 52.00 | 38.00 | 43.00 |
| | Leaf 6 | .. | 160.00 | 170.00 | 54.00 | 58.00 |
| Plant 2, | Leaf 1 | .. | 83.00 | 86.25 | 52.00 | 48.00 |
| | Leaf 6 | .. | 77.00 | 76.00 | 74.00 | 68.00 |
| Plant 3, | Leaf 1 | .. | 53.50 | 59.00 | 52.00 | 56.00 |
| | Leaf 6 | .. | 82.25 | 86.25 | 63.00 | 68.00 |
| Correlation coefficient | | .. | $r = 0.9956$ | | $r = 0.9145$ | |
| Determination coefficient | | .. | $R^2 = 0.9912$ | | $R^2 = 0.8363$ | |

Although differences are evident, statistical examination of the results show that in the case of iron ($R^2 = 0.9912$), the contents in the triangles follow very closely the patterns in the entire lamina. However, the data for manganese did not indicate a clear trend. The differences observed for manganese could be attributed to, (1) the possible occurrence of concentration gradients within laminae (i.e. from midrib to margin), and (2) the nonhomogeneous nature of hand crushed leaf samples. It should be possible to obtain more closer results if rectangular strips are taken instead of triangles from the laminae, and by powdering the material instead of hand crushing it.

### (iii) Investigations on toddy

The data presented in Fig. 3 show the weekly variation in the contents of iron, manganese, zinc and boron in toddy in two palms over a period of six months. Except, for zinc in which a fluctuating increase is noted with the onset of the dry season (February March), no consistent trend could be made out of the effect of season on micronutrient content.

It was also observed that *concentration* as well as the total *content* per collection of micronutrients varied considerably between palms. This was in striking contrast to what has been observed by Nathanael (1955) for nitrogen, phosphorus, calcium and magnesium where constancy in concentration was the feature.

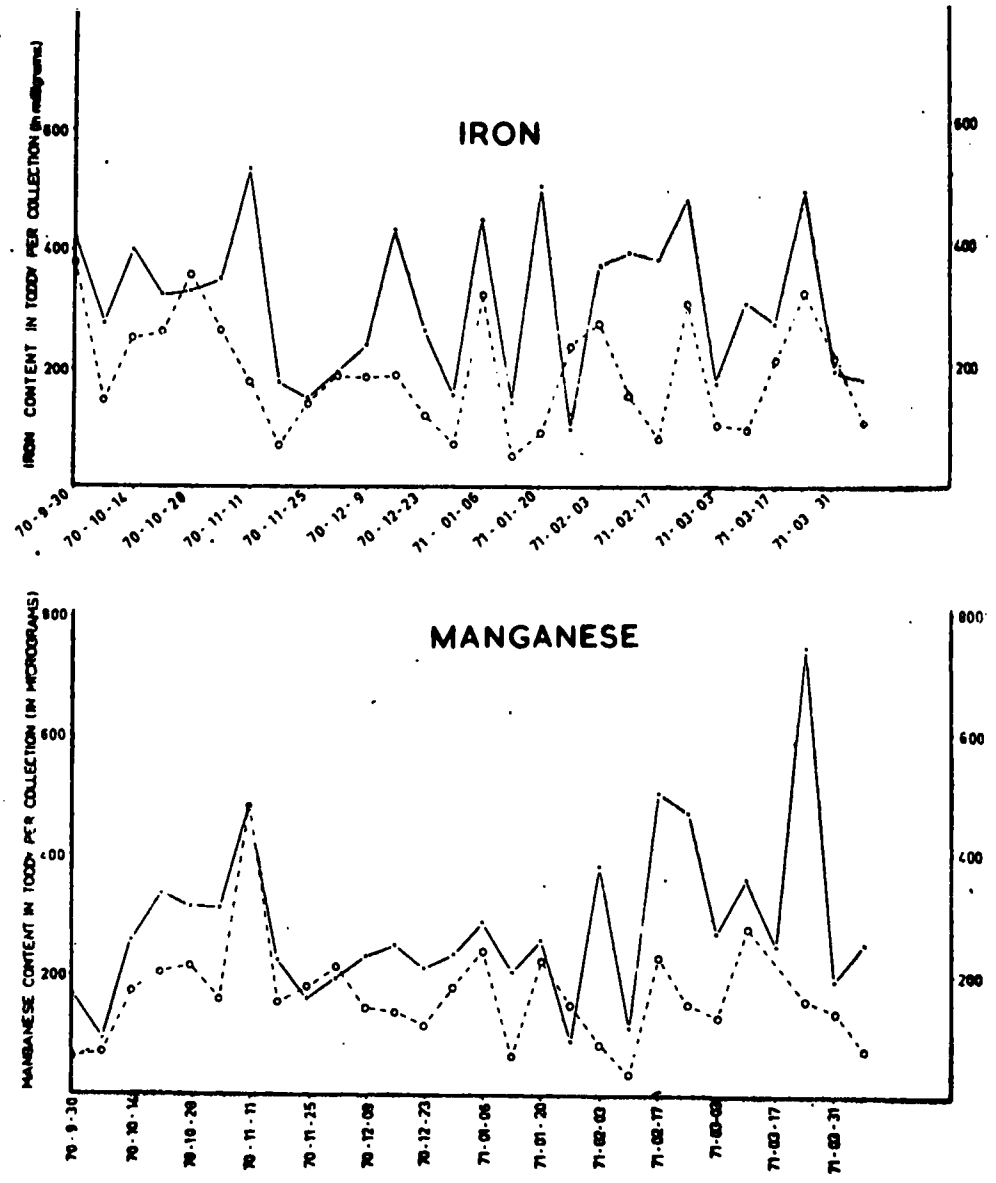



Fig. 3A. Weekly variation of Fe and Mn in the sap (toddy) of two palms during a period of six months.

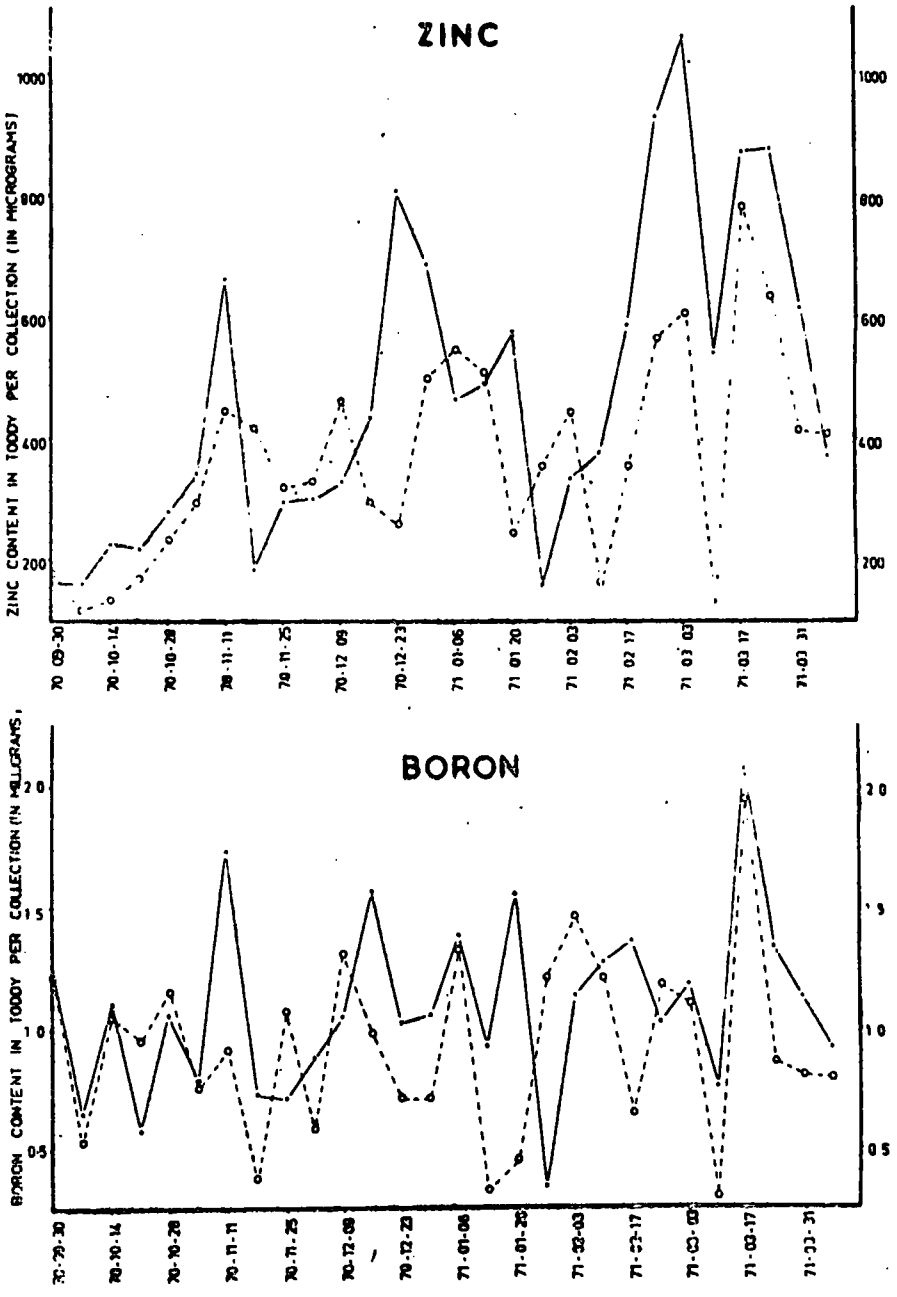



Fig. 3B. Weekly variation of Zn and B in the sap (toddy) of two palms during a period of six months.

In Fig. 4 is depicted the influence of season on diurnal variation of micronutrients in toddy. The plots in this figure represent the means of 6 palms, and the 6.00 a.m. curve in each graph represents the contents in the collection over a 12 hour period commencing at 6.00 p.m. the previous day. It is apparent that the content of micronutrients in toddy collected between 6.00 a.m. and 12 noon has been generally less than in that collected between 12 noon and 6.00 p.m.; a trend which has been consistent throughout the tapping period. A parallel increase in the volume of toddy has also been noted as the day advances. This is likely to be related to the increased rate of transpiration as the day passes over noontide.

On the whole toddy as a plant material can be considered to be unsatisfactory for nutritional studies on micronutrients, because of the irregular diurnal and seasonal fluctuations, and the wide variations between palms.

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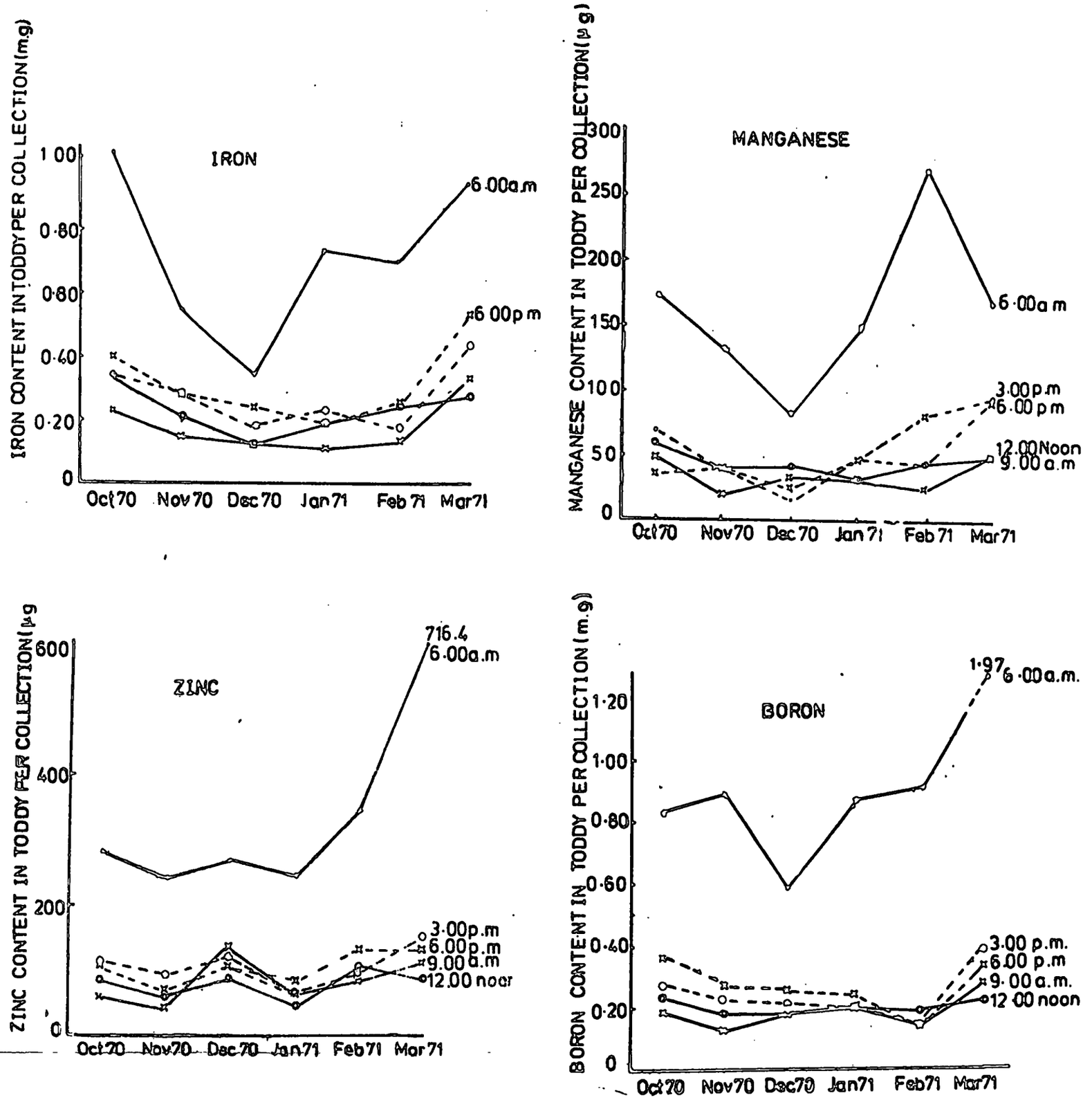


Fig. 4. Diurnal variation of Fe, Mn, Zn and B in the sap (toddy) of coconut palms during a period of six months.

(The plots represent the means of 6 palms, and the 6.00 a.m. curve in each graph represents the contents in the collection over a 12-hour period commencing at 6.00 p.m. the previous day).

## APPENDIX I

## PROCEDURE FOR THE DETERMINATION OF IRON, MANGANESE, COPPER AND ZINC IN PLANT MATERIALS

### OUTLINE OF METHOD:

Iron is determined by o-phenanthroline, manganese with periodate, copper by *bis*-cyclohexanone oxalyldihydrazone and zinc by dithizone on separate aliquots of a single plant digest.

### REAGENTS

Analytical-grade reagents are used, and solutions are made with deionised water.

**Sodium nitrite solution, 0.5%**

Prepare a fresh solution each day.

**Hydroxylamine hydrochloride solution, 2%**

**o-Phenanthroline solution, 0.1%**

Dissolve 0.1 g of o-phenanthroline in water by warming, and dilute to 100 ml.

**Ammonium acetate solution, 20%**

**Potassium periodate**

**Concentrated sulphuric acid**

**Concentrated nitric acid**

**Syrupy phosphoric acid**

**Ammonium citrate solution, 10%**

**Neutral red indicator solution, 0.001%**

**Sodium hydroxide solution, *N***

**Borate buffer solution**

Dissolve 12.2 g boric acid in water, add 11 ml 3*N* sodium hydroxide solution and dilute to 500 ml.

***bis*-Cyclohexanone oxalyldihydrazone solution, 0.25%**

Dissolve 0.25 g in 100 ml of 50% alcohol (v/v).

**Ammonium citrate solution, 2 *M***

Dissolve 486 g tri-ammonium citrate in 650 ml of water, add ammonia solution, sp. gr. 0.88 (approximately 25 ml) until the solution has a pH of 8.5 - 8.7, and dilute to 1 l. Extract solution with 10 ml portions of 0.1 % (w/v) dithizone solution in chloroform until the extractant remains green. Remove excess dithizone by repeated extractions with 50 ml portions of chloroform.

**Ammonia solution, 9 *M***

Mix equal volumes of ammonia solution, sp. gr. 0.88 and water.

**Ammonia solution, *M***

Add 56 ml ammonia solution sp. gr. 0.88 to water and dilute to 1 l.

**Ammonia solution, 0.01 *M***

Dilute 10 ml of approximately *M* ammonia solutions to 1 l.

**Carbon tetrachloride**

**Dithizone solution, 0.01 % (w/v) in carbon tetrachloride**

This reagent is stored in a refrigerator.

**Dithizone solution, 0.002% (w/v) in carbon tetrachloride**

Dilute 50 ml of above reagent to 250 ml with carbon tetrachloride. This reagent is prepared immediately before use.

**Hydrochloric acid, sp. gr. 1.18**

**Phenolphthalein indicator solution**

Dissolve 5 g in 50% alcohol (v/v).

**Sodium diethyldithiocarbomate solution, 0.125%**

This reagent is prepared immediately before use.

**Thymol blue indicator solution**

## STANDARD SOLUTIONS

### Iron solution

Dissolve 0.3511 g AR ferrous ammonium sulphate in water, add 5 ml 4*N* sulphuric acid and dilute to 500 ml (100 ug Fe++ per ml). Dilute 5 ml of above solution to 100 ml. 1 ml of this solution contains 5 ug of $Fe^{++}$.

### Manganese solution

Dissolve 0.2358 g AR $MnSO_4.4H_2O$ in water and make up to 500 ml. Dilute 40 ml of above solution to 100 ml. 1 ml. of this solution contains 40 ug of $Mn^{++}$.

### Copper solution

Dissolve 0.50 g pure copper foil in 5 ml concentrated hydrochloric acid and 1 ml of concentrated nitric acid. Boil to expel nitrous fumes and dilute to 500 ml (100 ug $Cu^{++}$ per ml). Dilute 1 ml of above solution to 500 ml. 1 ml of this solution contains 2 ug of $Cu^{++}$.

### Zinc solution

Dissolve 1.10 g of AR $ZnSO_4.7H_2O$ in water, add 2 ml HCl (sp. gr. 1.18), and dilute to 250 ml (100 ug Zn per ml). Dilute 3 ml of above solution to 1 litre. 1 ml of this solution contains 3 ug Zn.

## PROCEDURE

### Preparation of extract

Weigh 2.0 g of oven dried plant material into a pyrex 50 ml conical flask or beaker. Ash overnight in a muffle furnace at 450° C. Add 10 ml of 2.0 *M* HCl, 1 ml of 0.5% sodium nitrite solution and boil for 10 minutes. Cool, dilute to 20 ml, mix well and leave overnight for silica to settle out.

### Estimation of iron

Pipette 1 ml of the plant ash extract into a 10 ml graduated flask. Add 2 ml of 2% hydroxylamine hydrochloride, 2 ml of 0.1% o-phenanthroline reagent and 2 ml of 20% ammonium acetate solution. Dilute to 10 ml and after 1 hour measure optical density of solution in a 1 cm cell at 510 mu. Construct calibration curve relating optical density to amounts of 5–25 ug of iron.

### Estimation of manganese

Take 10 ml of extract in a 50 ml beaker, add 3 ml of concentrated $H_2SO_4$ and heat gently on a hot plate for 20 minutes to remove chlorides. Cool, add 1.5 ml of concentrated nitric acid and 1.5 ml of syrupy phosphoric acid and dilute to about 20 ml. Add 0.1 g of potassium periodate and boil for 10 minutes. After cooling transfer solution to a 25 ml graduated flask and dilute to volume. Measure optical density in a 1 cm cell at 525 mu. Construct standard graph by carrying amounts of manganese 40–200 ug through the above procedure.

### Estimation of copper

Pipette 4 ml aliquot into a 10 ml graduated flask. Add 2 ml 10% ammonium citrate solution and 0.5 ml of 0.001% neutral red indicator solution. Neutralise with *N* NaOH solution till just yellow. Add 1 ml borate buffer solution and 0.2 ml of *bis*-cyclohexanone oxlyldihydrazone solution, and dilute to 10 ml. After standing for 1 hour measure optical density in a 1 cm cell at 595 mu. Prepare calibration curve by relating optical density to amounts of copper 2–10 ug through the above procedure.

### Estimation of zinc

Transfer 3 ml of plant ash extract to a 100 ml separating funnel and dilute to approximately 25 ml. Add 2.5 ml 2 *M* ammonoium citrate solution, a few drops of thymol blue indicator solution and HCl until just red. Add 9 *M* ammonia solution dropwise until red colour begins to turn yellow (pH 2.5). Add 10 ml 0.01 % dithizone solution shake for 1 minute and allow the layers to separate. Discard the lower layer. Add 5 ml of carbon tetrachloride, shake for 15 seconds and allow the layers to separate. Discard the lower

layer. Repeat with 5 ml carbon tetrachloride and allow any carbon tetrachloride remaining on the surface to evaporate. Add a few drops phenolphthalein indicator solution and add 9 *M* ammonia solution until just pink. Add 5 ml sodium diethyldithiocarbomate solution and exactly 25 ml 0.002% dithizone solution. Shake vigorously for 1 minute and allow the layers to separate. Immediately transfer the bulk of the lower layer to another separating funnel, add 50 ml of 0.01 *M* ammonia solution, shake vigorously for 30 seconds and allow layers to separate. Without delay and avoiding direct sunlight, filter the lower layer into a dry test tube and measure the optical density in a 1 cm cell at 540 mu.

Construct calibration curve by carrying amounts of zinc 3–15 ug through the above procedure.

## REFERENCES

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## APPENDIX II

## PROCEDURE FOR THE DETERMINATION OF BORON IN PLANT MATERIALS

### REAGENTS

**Dianthrimide reagent, 0.03%**

Dissolve 0.6 g dianthrimide in 100 ml 98% sulphuric acid. Dilute 5.0 ml of solution to 100 ml with 98% sulphuric acid.

**Standard boron solution**

Dissolve 1.426 g AR boric acid in water and dilute to 500 ml. 1 ml of this solution contains 500 ug B Store all reagents in soft glass or boron-free glassware.

### PROCEDURE

Ash 0.5–1.0 g of oven dried plant material in a silica dish overnight at 450° C, dissolve in 10 m 2 *N* $H_2SO_4$ and filter through Whatman No. 40 paper into a soft glass tube. Pipette 1 ml into a stoppered soft glass bottle (30 ml capacity) and add 10 ml 0.03% dianthrimide reagent from a burette. Heat solution in an oven at 90° C for 3 hours, cool in a desicator and measure optical density after 1 h in 1 cm cells at 620 mu.

Prepare calibration curve by relating optical density to amounts of boron 0–4 ug through the above procedure. Solution should contain the appropriate amount of B in a volume of 1 ml in order that the concentration of $H_2SO_4$ remains constant throughout.

## REFERENCES

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