

ON THE DETERMINATION, AND CONCENTRATION
" OF ZINC IN PINEAPPLE PLANTS AND SOILS

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By
Clarence Lyman

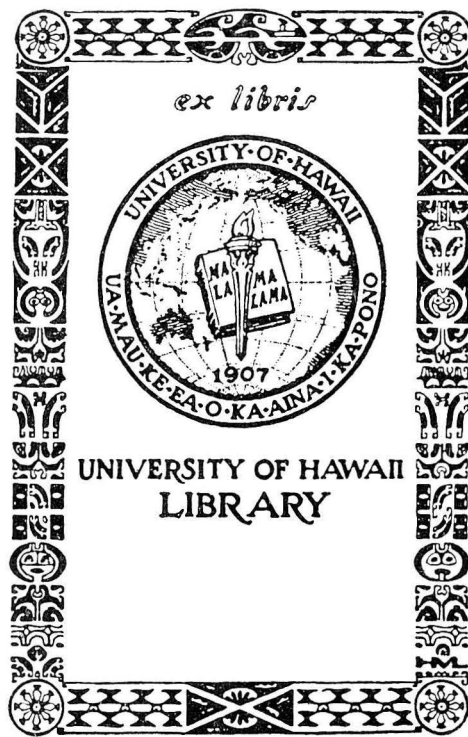
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PREFACE

In modern agriculture at least fourteen elements are considered to be indispensable for plant growth. They are frequently divided into two groups. The first includes those elements required in quantities which are easily measured and studied, and are: Carbon, Hydrogen, Oxygen, Nitrogen, Phosphorus, Potassium, Sulfur, Calcium, Iron, and Magnesium. The second group is termed the minor or, more recently, the micro-nutrient elements. They are no less essential but are required in such small amounts as to make their functions relatively uncertain. Copper, Zinc, Boron, and Manganese and possibly others comprise this group.

The role of zinc in plant growth is still a controversial subject. This status is largely the result of difficulties in quantitatively determining the small amounts involved—amounts on the order of a fraction to several hundred parts per million.

Of particular interest was the discovery that certain abnormal conditions of crops could be corrected by applications of zinc compounds. Experiments in Hawaii have shown that a mottling and curvature of leaves, and some of the failure to fruit exhibited by pineapple plants could be remedied by means of zinc fertilization in the form of sprays applied to the foliage.

There remains the question of the relationship between the available soil zinc and the concentration of this nutrient in plants necessary for normal growth. This can be answered only by quantitative micro-analyses of the various plant parts and of soil extracts.

The pineapple plant affords an ideal means of approaching the problem for two reasons: its already known growth symptoms which respond to zinc-sulfate fertilization; and the readiness with which the plant parts

may be segregated.

The recently developed Polarograph furnishes a convenient means of determining small quantities of zinc in the presence of most of the anions and cations occurring in plants and soils. It was used in making this study and its adaptability as a standard instrument for quantitative analysis is amply demonstrated.

As modifications of existing polarographic analytical methods were found to be necessary, this paper deals with: first, an original method for the determination of heavy metals, especially zinc, in plant materials and soil extracts; and secondly, an attempt is made to correlate the readily available zinc in several pineapple soils, the amounts absorbed by plants growing in them, and their growth symptoms as observed in the field.

THE DETERMINATION OF ZINC IN PLANT TISSUES AND SOILS

Introduction.

Numerous chemical procedures for determining small quantities of zinc have been developed, involving most of the common types of quantitative analysis. Some of these have been successfully used for many years.

Bertrand and Javillier (9) (10) determined the zinc content of many plants gravimetrically by means of the calcium-zincate isolation which they devised. This procedure required very large samples, but was used extensively with satisfactory results.

The volumetric ferro-cyanide method was used as early as 1879 by Mylius (34). By matching turbidities resulting when standard and unknown zinc concentrations were mixed with a potassium ferro-cyanide solution, Mylius reported concentrations as low as nine parts per million in a study of spring waters. A similar ferro-cyanide method requiring 25 gram samples is proposed by the A. O. A. C. (4) for plant materials.

Boggs and Alben (11) determined zinc nephelometrically on zinc ferro-cyanide suspensions, but Lott (32) found that the colloidal zinc ferro-cyanide was unstable, resulting in wide fluctuations in electrophotometric measurements of the reflected light.

Cone and Cady (18) adapted the zinc-potassium ferro-cyanide reaction to direct titrations by introducing diphenyl-benzidine as an internal indicator. An iodometric method was proposed by Hibbard (26) in which zinc was titrated after a ferro-cyanide-oxalate separation.

Todd and Elvehjem (42) proposed the determination of zinc by precipitating it as zinc ammonium phosphate and subsequently determining the phosphate colorimetrically.

Lott (32) adapted a hydrogen sulfide method of separating zinc from

solutions, and subsequently determined it as the reduced salt of 5-nitroquinaldic acid.

Since 1933, when Wölbling and Steiger (44) published studies of the reactions of dithizone (diphenyl-thio-carbazone), it has come into common use as an extracting agent for the heavy metals. Fischer (20) worked with this reagent and presented methods of qualitative and quantitative analyses and described the complex reactions occurring in its use. Sandell (40) adapted this work to the colorimetric determination of copper, zinc, and lead in silicate rocks. Hibbard (27) proposed a similar method for the measurement of small quantities of zinc in plant materials, later (28) he extended this procedure to the determination of zinc, cobalt, and nickel.

Spectrographic methods of analysis have been found to lack sufficient sensitivity for the micro-analysis of zinc. Vanselow and Laurance (43) used a grating spectrograph but suggested the possibility of a greater sensitivity with a prismatic type. However, Rogers and Gall (39) were able to determine small amounts only by using special purifications and precautions which they considered superfluous in view of simpler methods in existence. Ballard (5) set as the lower limits for zinc, by ordinary methods of analysis with the quartz prism spectrograph, the range of 100 to 1,000 parts per million.

In 1938 Stout, et al. (41) proposed a polarographic method for the estimation of zinc in dry-ashed material, in the presence of cadmium, lead, nickel, copper, cobalt and bismuth, after a dithizone separation of these from iron, aluminum, manganese, calcium, magnesium and the alkali metals.

Reed and Cummings (38) described a method for polarographically determining zinc in the presence of all plant constituents excepting iron and aluminum. The plant material was dry ashed, taken up in hydrochloric acid, the iron and aluminum precipitated by adjusting the pH to

between 4 and 5, and the solution filtered. The zinc in the filtrate was then determined by the method of Stout, et al. (41).

Preparation of solutions from plant tissue for the determination of zinc by the polarographic method.

At the outset of this study the reliability of the polarographic method proposed by Stout, et al. (41) for the determination of zinc was investigated in regard to its adaptability. Of particular interest was its use as a rapid procedure for the determination of zinc in small samples.

The essential features of the method as used are as follows:

One gram samples of dry tissue were ashed at 400° C. Taken up in hydrochloric acid and diluted to 100 ml., a suitable aliquot of the supernatant liquid was pipetted into a 250 ml. conical separatory funnel containing 5 ml. of 10 per cent ammonium citrate. The solution was then adjusted to a pH between 8 and 9 with ammonium hydroxide. The heavy metals, including zinc, were then extracted by means of a chloroform solution of dithizone.

The chloroform was evaporated off, and the dithizonates destroyed by heating with a mixture containing 3 ml. of concentrated nitric acid and 0.5 ml. of perchloric acid. The salts were converted to chlorides by three evaporations with dilute hydrochloric acid and heating for a few minutes at a high temperature.

The residue was taken up in 2.0 ml. of a solution 0.025 N with respect to potassium-thiocyanate and 0.10 N with respect to ammonium acetate adjusted to pH 4.6. The zinc was then determined polarographically.

A consideration of preliminary results obtained by the above procedure showed them to be unreliable. This was especially pronounced when attempts were made to recover increments of zinc added to the tissue before ashing. Further, the method was found to be cumbersome and time consuming. The unreliability of results was probably affected by the following factors: (1) the occlusion of zinc within the siliceous materials fused upon ashing, as suggested by Piper (37) and Gieseking et al. (22); (2) the incomplete separation of zinc by the dithizone procedure of

extraction; and (3) the volatilization of metals in the ashing procedure. To eliminate the difficulties apparent in dry ashing, the following wet ashing procedure was adopted.

A one gram sample of plant tissue and 15 ml. of concentrated nitric acid were placed in a 200 ml. high form beaker, covered with a watch glass, and digested at low heat until the initial rapid oxidation subsided. The heat was then increased to boiling and continued until near dryness; the beaker cooled, and 10 ml. of a 6:4 nitric-perchloric acid mixture added. The digestion was resumed, allowing the perchloric acid to reflux for 3 or 4 hours, thus ensuring a complete oxidation of the waxes. The beaker was then cooled, 1 ml. of hydrochloric acid added and allowed to reflux for a few minutes, thus dissolving any entrained material deposited upon the watch glass and beaker walls.

After cooling, the watch glass and beaker walls were washed with water and the contents taken to dryness. The last traces of perchloric acid were then driven off by brushing the beakers with a bunsen-flame.

The residual salts were converted to chlorides by three successive evaporations with 5 ml. portions of 10 N hydrochloric acid.

Next, attempts were made to eliminate the dithizone extraction since it was found to be cumbersome and, in some instances, incomplete. The plant residues, prepared as above, were dissolved in hydrochloric acid and the silica removed by centrifuging.¹ Instead of using the dithizone extraction at this point, the solution was evaporated to dryness and the residue dissolved in the indifferent electrolyte (0.1 N ammonium acetate at pH 4.6 and 0.025 N with respect to potassium thiocyanate). Experience showed that some of the residues treated in this way formed clear solutions which could be directly applied to the polarographic method and the zinc determined. However, residues from certain tissues could not be completely dissolved, and distinctly turbid solutions resulted. Also, an incomplete recovery of the zinc was found. Tests indicated that the high phosphate

¹ The use of filter paper was avoided since it was found that the best obtainable grades either added to or decreased the zinc concentration in the filtrate. This is in agreement with the findings of Hibbard (29).

content of the residues (1.07 to 3.63 mgm.) was apparently responsible for the insolubility of certain of the compounds in the indifferent electrolyte.

Since the removal of the phosphate from residues of the perchloric acid digestion seemed to be necessary in order to eliminate the dithizone extraction, studies of the removal of phosphate were undertaken. At the outset, the removal of phosphate by precipitation with zirconium was investigated. By this means sufficient phosphate could be removed to enable the complete solution of the residues in the indifferent electrolyte, but apparently the excess zirconium made polarographic determinations unsatisfactory.

Further studies on the removal of phosphate from the residue of the perchloric acid digestion showed that it could be eliminated by absorption with meta-titanic acid. Tests were then made of the effects of meta-titanic acid upon the composition of the solutions to be analyzed. As the titanic acid is practically insoluble in dilute organic acids, acetic acid was used to dissolve the digested plant residues (see page 6). The following procedure was then applied for the removal of the phosphate by meta-titanic acid:

The residue from the perchloric acid digestion was taken up in 10 ml. of 0.5 N acetic acid, refluxed for 10 minutes, cooled, and the watch glass removed after rinsing. The beaker was thoroughly rinsed and polished to suspend the silica. About 0.2 of a gram of finely pulverized meta-titanic acid was added and the suspension intermittently swirled for an hour.

The suspension was transferred to a graduated, pyrex centrifuge tube and made up to the 40 ml. mark with water. The silica and titanic acid were then removed by centrifuging. An aliquot of 25 or 35 ml. of the supernatant liquid was syphoned into a 50 ml. erlenmeyer flask and taken to dryness overnight by heating below boiling on a hotplate. To ensure the complete removal of acetic acid

from the flask neck, it was placed in an 80° C. oven for 30 minutes.

This residue was dissolved in measured amounts of an indifferent electrolyte 0.1 N with respect to acetic acid adjusted to pH 4.6 with ammonium hydroxide, and 0.050 N with respect to potassium thiocyanate. Dissolution was aided as required by using a pyrex stirring rod.

After complete solution, the zinc was determined polarographically.

Phosphate determinations made on the solutions prepared as above showed that from 95 to 100 per cent had been removed. Tests with standard solutions showed that there were no losses of zinc by adsorption or occlusion with the meta-titanic acid.

The significance of the use of meta-titanic acid for the removal of the phosphate is well illustrated by the following test. Four one gram portions of each of two samples of pineapple tissue were treated according to the wet-ashing procedure described on page 6, after adding ten gammas of zinc to two of each group. One half of the samples were treated according to the phosphate removal procedure described and the other half was taken up directly in the indifferent electrolyte without removing the phosphate. The zinc concentrations of these solutions were then determined by the polarographic method. The amounts of zinc calculated from the polarograms are tabulated in Table I.

It may be seen from an inspection of Table I that in all cases there was more zinc in the solutions which were freed of phosphate than in those which had not been treated. Further, when the phosphate was not removed, there was only a 60-70 per cent recovery of the 10 gammas of zinc added to the samples.

Consequently, by using the procedure for phosphate removal, consistent replications and complete recoveries of added zinc were found possible. Thus a procedure was developed which did not require the sepa-

Table I. Determination of zinc in pineapple plant tissues. A comparison of the zinc recovered when determined after removing and not removing the phosphate.

Sample No.	Procedure	Zinc added	Zinc found	Recovery of added zinc
		gammas	gammas	per cent
I (E ₁)	PO ₄ removed	None	45	
"	" "	10	55	100
"	PO ₄ not removed	None	44	
"	" " "	10	50	60
VIII (E ₁)	PO ₄ removed	None	15	
"	" "	10	25	100
"	PO ₄ not removed	None	14	
"	" " "	10	21	70

ration from iron, aluminum, manganese, calcium, magnesium, and the alkali metals by means of dithizone (41); or the removal of the iron and aluminum by pH adjustments and filtration, as done by Reed and Cummings (38).

This analytical procedure was found to be rapid and readily usable in routine determinations of zinc in plant materials. Although the nickel and lead were not determined in this study, these may readily be estimated from the same polarograms as the zinc.

Preparation of soil extracts for the determination of zinc by the polarographic method.

The following procedure was found to be satisfactory for obtaining some measure of the available zinc in soils. It was found to be well adapted to the routine examination of pineapple soils.

The soil extract was prepared by placing a 10.00 gram sample of 10 mesh soil and 200 ml. of 1.0 N acetic acid, adjusted to pH 4.6 with ammonium hydroxide, in a suitable pyrex flask and mechanically shaking for two hours. After standing overnight, the supernatant liquid was transferred to a 250 ml. centrifuge bottle and clarified by centrifuging.

A 150 ml. aliquot of this extract was placed in a 200 ml. beaker, taken to dryness below boiling, and freed of extraneous acid by cooling, then twice washing down the beaker walls and evaporating to dryness. The beaker was covered with a watch glass, cooled, 10.0 ml. of a 9:1 nitric-perchloric acid mixture added, heated to boiling, and allowed to reflux until nearly dry. The beaker was then cooled, 1.0 ml. of hydrochloric acid added, and allowed to reflux for a few minutes, thus dissolving any entrained material deposited upon the watch glass and beaker walls. After cooling, the watch glass and the beaker walls were washed with water and the contents taken to dryness. The last traces of perchloric acid were removed by brushing the beakers with a bunsen-flame.

The residual salts were converted to chlorides by three successive evaporations with 5 ml. portions of 10 N hydrochloric acid. The chlorides were taken up in 0.5 N acetic acid, heated slightly, and the beaker thoroughly polished. The suspension was transferred to a graduated pyrex centrifuge tube and made up to the 25 ml. mark with water. The silica was removed by centrifuging. An aliquot of 20.0 ml. was syphoned into a 50 ml. erlenmeyer flask and taken to dryness overnight by heating

below boiling on a hot plate. The removal of acetic acid was ensured by placing the flask in an 80°C oven for a few minutes.

The residue was taken up in 6.0 ml. of the nonpolar electrolyte, and the solution analyzed polarographically.

The acetic acid - ammonium acetate mixture was decided upon as an extracting agent because the large quantities required could be prepared very readily from reagents easily freed of heavy metals by redistillation. Since other workers (29) (31) have shown that insignificant amounts of zinc are removed by neutral extracting reagents, a buffered solution was used.

Reagents.

Throughout this study especially purified reagents or those with known, low zinc contents were used. In all cases the chemicals were polarographically examined for their heavy metal content.

The reagents used in the analysis of plant materials and soil extracts are:

1. Double distilled water prepared in a pyrex glass still and found to be free of heavy metals. This was used in all instances requiring water.
2. Concentrated nitric acid, C. P. grade, selected for its low zinc content (0.065 gamma zinc per ml. acid).
3. 70-72% Perchloric acid, C. P. grade, selected for its low zinc content (0.139 gamma zinc per ml. acid).
4. Hydrochloric acid, redistilled in pyrex glass and checked for its blank. (0.000 gamma zinc per ml.).
5. Acetic acid, redistilled in pyrex glass and checked for its blank. (0.000 gamma zinc per ml.). Diluted as required.
6. Ammonium hydroxide, redistilled in pyrex glass and checked for its blank. (0.000 gamma zinc per ml.). Diluted as required.
7. Potassium-thiocyanate, C. P. grade, selected for the absence of heavy metals (0.000 gamma zinc per gram).
8. Meta-titanic acid, prepared from titanium-potassium oxalate as follows:

One hundred grams of titanium-potassium oxalate were dissolved in water, filtered into a two liter beaker, and 500 ml. of concentrated sulfuric acid added. The solution was boiled until the oxalic acid was partly decomposed and carbonized. The oxidation was completed by adding a few drops of concentrated nitric acid and continuing the boiling until a clear yellow color was obtained and the NO_2 fumes ceased evolving. Excessive boiling should be avoided. ²(Excessive boiling results in the formation of difficultly soluble higher sulfates.)

The solution was cooled and diluted to three liters with distilled water, ammonium hydroxide added until the precipitated ortho-titanic acid could be dissolved only with extended stirring. A mixture of 220 ml. of glacial acetic acid and about 180 ml. of ammonium hydroxide was added and the acidity finally adjusted to pH 4.2.

The entire mixture was then diluted to eight liters, brought rapidly to boiling, and kept in ebullition for 30 minutes to precipitate the meta-titanic acid. The floccules were allowed to settle and the clear solution syphoned off.

The titanic acid was washed by alternately centrifuging and dispersing it, using dilute acetic acid in the first rinsings and double distilled water in the final. The washed precipitate was dried at 70°C until it could be pulverized in a mortar.

ZINC DEFICIENCY IN PINEAPPLES IN RELATION TO PLANT AND SOIL COMPOSITION

Introduction.

The early studies of the role of zinc in relation to plant growth showed that the application of zinc compounds to various crops growing in water cultures or soils caused a toxicity in some instances and a stimulation in others. This was found to vary with the species of plants and with soil type. For example, Nakamura (35) and Brenchley (12) observed these effects and considered zinc to be a plant poison. On the other hand, Javillier (30), and later Allison et. al. (3), obtained definite growth improvements by several crops fertilized in the field with zinc sulfate at rates between one and ten pounds per acre.

The relative insolubility of the soil zinc compounds and the fixation of zinc by soils was recognized at an early date. The ability of soils to fix zinc from solutions was demonstrated by von Gorup-Besanez (23) in 1863, while the exchange reaction between zinc sulfate solutions and soils was studied by Fretag (21) in 1868. Furthering this work, Baumann (8) showed that the fixation ranged from a maximum in highly organic soils to a minimum in sandy soils, with clays and calcareous soils between these limits. As the compounds responsible for this fixation, Baumann listed: "humic-acids" (alpha humus), zeolites, aluminum hydroxide, and calcium and magnesium carbonates. Later, Jones et. al. (31) added phosphates to this list.

The importance of the zinc supplying power of soils was convincingly demonstrated when Chandler and co-workers (14) undertook a study of the physiological disease of deciduous fruit trees known as little-leaf or rosette. In 1931 they reported that certain trees exhibited definite signs of recovery when treated with iron sulfate which was highly

contaminated with zinc. In the ensuing year they (15) showed that this response was actually a result of the zinc present rather than the iron alone. Almost simultaneously Alben et. al. (1) in Louisiana, and Finch (19) in Arizona, found that zinc fertilization brought about a recovery by pecans from a similar little-leaf condition.

It has since been shown that the following, similar physiological diseases can frequently be overcome by treatment with zinc compounds: "white bud" of cereal crops (7), "bronsing" of tung trees (33), "yellows" of walnuts (24), "frenching" and "mottle-leaf" of citrus trees (13), and "little-leaf" of grape vines, stone fruits, and other trees (15) (16) (17).

Several workers (17) (25) (31) have shown that not all species of plants affected by the above symptoms are equally restored by the same compounds of zinc or by the same means of application. This may account for the failure of some workers (3) (6) (14) to overcome the disease when testing the effects of a specific form of zinc applied in a single method. It is also conceivable that the greater portion of the zinc applied to foliage or soils is never absorbed by the plants.

The leaves of pineapple plants growing in various parts of Hawaii have, on occasion, shown symptoms of physiological disorders which have been found to be correctable by spraying with zinc sulfate. There are apparently two phases of this disorder, the more common and less severe being a mottling and blistering of the upper surfaces of the leaves. This is illustrated in Figure 1. On occasions, the younger leaves of the heavily mottled plants are unmottled, but show a marked curvature. This affecting of the younger leaves apparently occurs as a secondary symptom of zinc deficiency. Severe curvature is illustrated in Figures 2 and 3, and normal, uncurved "heart" leaves in Figure 4. Plants which exhibit this leaf-curvature do not produce fruits or secondary growth without considerable delay.

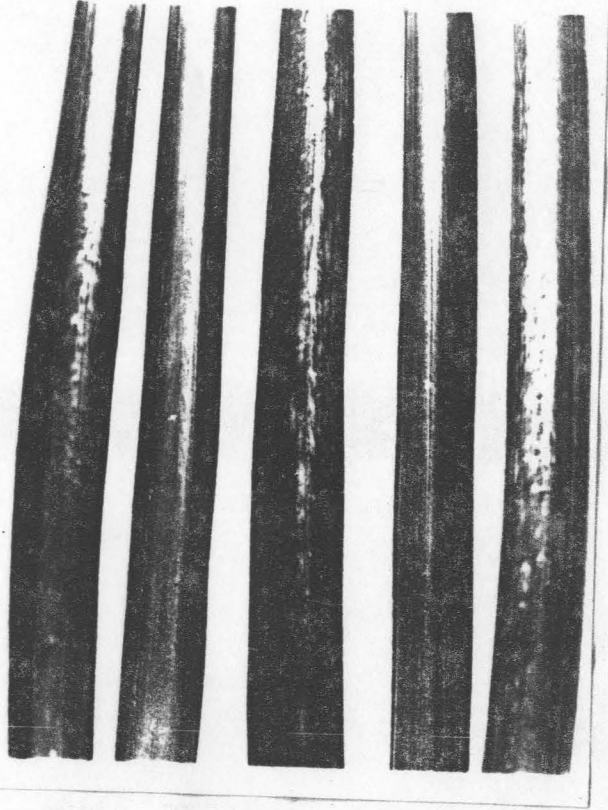


Figure 1. The mottling and blistering of upper surfaces of pineapple leaves which have been overcome by spraying with solutions of zinc sulfate.

From left to right, the leaves are alternately mottled and unmottled.



Figure 2. Severe curvature of pineapple (heart) leaves. A secondary phase of the physiological disease illustrated in Figure 1. Normal growth (Figure 4.) is resumed after spraying with zinc sulfate.

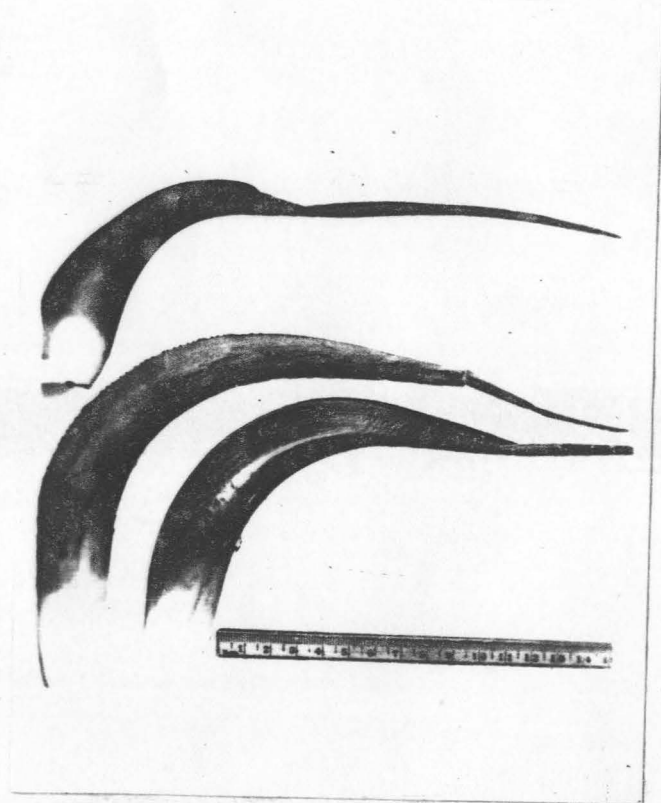


Figure 3. Severely curved pineapple leaves. Note the absence of mottling or blistering characteristic of the earlier phases of this disease.

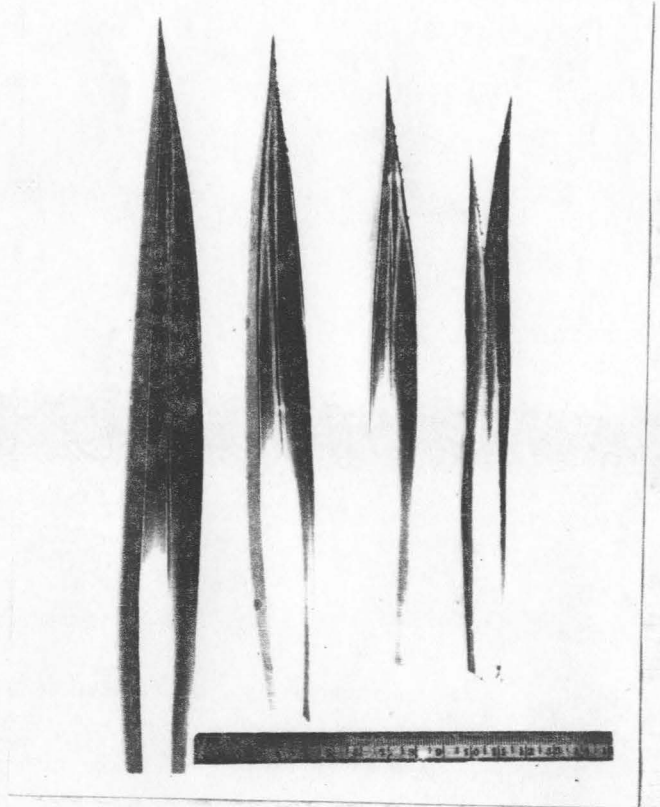


Figure 4. Normal pineapple (heart) leaves.

In order to study the relationships of zinc in pineapple soils, the zinc absorbed by the plants growing in them, and the symptoms of zinc deficiency exhibited, collections were made of plant and soil samples covering as wide a range of variations as possible. The following empirical classification of the appearance of the plants was adhered to as closely as possible in describing the samples taken for the purposes of this investigation.

<u>Zinc deficiency index</u>	<u>Plant appearance</u>
0	Normal.
1	<u>Slight mottling</u> : Appearing as a symptom of incipient deficiency which often disappears with further growth. It may be incident only after blossoming.
2	<u>Medium mottling</u> : A significant deficiency usually incident before blossoming.
3	<u>Heavy mottling</u> : Often incident before the red-bud stage.
4	<u>Very heavy mottling</u> : Usually incident in the initial stages of growth shortly after planting, causing a delayed fruiting. Fruits may be abnormal.
5	<u>Curvature of leaves</u> : Appearing after the very heavy mottling stage described in 4, above. Fruiting is greatly delayed.

Several workers (6) (7) (25) (31) (36), have indicated that symptoms of zinc deficiency appear at various stages of growth and become more pronounced under certain weather conditions which have been associated (17) with the metabolic status of the plants. Similar variations of the incidence of deficiency symptoms have been noted with pineapples. Thus, wherever possible, the classification of the samples was based on the knowledge of pre-

vious as well as current crop cycles. Where little knowledge of the previous cycles was available, the samples were of necessity judged by the appearance of the plants in the area at the time of sampling.

To avoid misrepresentation, all soil and plant samples were taken from areas which had received no zinc fertilization. Where plant samples were taken and composited, the corresponding soil samples were obtained and composited from the positions immediately under the plants when removed. Soil samples were also taken from fallow areas where symptoms of zinc deficiency were known to have occurred during the previous crop. All soil samples were taken from 0 to 12 inches deep.

Soil composition and zinc deficiency symptoms.

The soil samples which were collected for the purposes of this study were air-dried; roots, leaves and rocks were removed; and the material pulverized to pass a 10-mesh sieve.

Soil zinc determinations were made by means of the polarographic procedure previously described and are expressed on the oven dry basis after subtracting the reagent blank of 0.06 gamma. Soil acidities were determined with a glass electrode. The results of these determinations are listed in Table II.

An inspection of Table II shows an interesting relationship between the soil zinc, as determined, and the indices of deficiency exhibited by the crops. The only discrepancy is that shown by soil sample 5 which showed a large amount of available zinc although the plants growing on it appeared to be zinc deficient. It is possible that the observed deficiency symptoms were from causes other than a deficiency of zinc. On the other hand this soil sample might have become contaminated or possibly the particular extracting solution chosen for measuring the available zinc is not

Table II. Showing the relationship of zinc extracted from soils, by N acetic acid adjusted to pH 4.6, and the indices of zinc deficiency exhibited by pineapple plants growing in these soils.

Sample No.	Soil sample source	Soil pH	Zinc deficiency index	Zinc determined p.p.m.
12	T. Yonamine, So. Halawa, Oahu	4.9	0	3.5
14	Kauai Pine., Hanapepe, Fd. 39C	5.6	0	3.1
2	Libby, Waipio C, Oahu, Fd. 137	4.1	0	2.6
13	Kauai Pine., Kalaheo, Fd. Y	4.5	0	2.2
10	K. Tokuhara, Aiea Hgts., Oahu	<u>4.3</u>	0	<u>1.7</u>
		mean <u>4.7</u>		mean <u>2.6</u>
15	Kauai Pine., Hanapepe, Fd. 39C	5.6	1	1.5
11	Y. Yonashiro, Aiea Hgts., Oahu	4.3	1	0.8
17	Hawn. Pine., Halemanu, Oahu	<u>4.0</u>	1	<u>0.7</u>
		mean <u>4.6</u>		mean <u>1.0</u>
1	Libby, Waipio C, Oahu, Fd. 137	4.0	2	1.4
6	Trop. Frt. Ex.*, Red Hill, Oahu	5.4	2	1.0
19	Libby, Waipio C, Oahu, Fd. 137	<u>4.7</u>	2	<u>0.9</u>
		mean <u>4.5</u>		mean <u>1.1</u>
5	Trop. Frt. Ex., Red Hill, Oahu	5.9	3	3.9
4	Trop. Frt. Ex., Red Hill, Oahu	4.4	3	0.8
3	Trop. Frt. Ex., Red Hill, Oahu	<u>4.3</u>	3	<u>0.6</u>
		mean <u>4.9</u>		mean <u>1.8</u>
7	S. Tawata, Kalaheo, Kauai	<u>4.4</u>	4	<u>0.6</u>
16	Kauai Pine., Omac, Fd. M	4.3	5	0.6
9	Kauai Pine., Omac, Fd. M	5.5	5	0.6
8	S. Tawata, Kalaheo, Kauai	4.3	5	0.5
18	Libby, Kunia, Oahu, Fd. 134	<u>4.2</u>	5	<u>0.5</u>
		mean <u>4.6</u>		mean <u>0.6</u>

* Tropical Fruit Exchange, Red Hill, Oahu

universally applicable. This soil was the least acid of any of those studied and the effect of a highly buffered acid extracting solution may have given unreliable results. This point needs further investigation. However, it is significant to note that plants showing no zinc deficiency symptoms were found growing in soils having more than 1.7 parts per million of zinc extracted with 1.0 N acetic acid, adjusted to pH 4.6 with ammonium hydroxide.

Although it was found that in some extracts there were also measurable quantities of lead and nickel, this study was confined solely to zinc, and the possible significance of other heavy metals was not considered.

Plant composition and zinc deficiency symptoms.

The pineapple plants in each set were arbitrarily broken into groups* of comparable development and composited. Suckers were treated as mother plants. All leaves which had been fully or partially grown at the planting time (classed as "A" and "B" leaves) were discarded, and the remaining leaves segregated as follows:

"C"—Fully grown leaves at an angle of more than 45 degrees from the vertical;

"D"—The longest fully grown leaves, or those between 30-45 degrees from the vertical;

"E"—The leaves more than a foot in length and less than 30 degrees from the vertical;

"F"—The remaining leaves and leaf primordia.

Each group of leaves was then divided into three portions as follows:

(1) the basal, white meristematic tissue; (2) the central portion or that

* This system was adopted upon the suggestion of Dr. C. P. Sideris of the P. P. C. A. Experiment Station.

between the white meristematic tissue and the region where the leaves begin to taper to a point; and (3) the remaining portions or tips. The fragments of leaf meristem were pared from the stumps and the top inch cut off and considered to be the growing point.

Leaves of fruiting plants were segregated as above but the last formed leaves were placed in the "E" rather than the "F" group. These included the leaves on the peduncle. Only the shells of fruits were kept for analysis. Two methods were used to segregate secondary growth. In sets I and V all crowns, slips, and suckers were composited, while in set VI the crowns were analyzed separately.

All samples were cut with a knife into narrow strips and dried in a forced draft dryer at 70° C. They were then milled to pass a one millimeter mesh screen. After thoroughly mixing, the milled samples were bottled and placed in a 70° C oven overnight, removed, tightly stoppered, and stored.

The zinc in the plant tissues was determined by means of the polarographic procedure previously described and presented in Table III. An examination of these data shows that in general the highest concentrations of zinc in the growing points appears to be only one which bears a close relationship to the observed deficiency symptoms. The possible exception to this is in the instance of the very severely zinc deficient plants (Plants VIII and IX), where there was found a considerably lower amount of zinc in all meristematic tissues.

If it had been possible to have studied plants which were of more nearly comparable ages and had grown under similar environmental conditions, a more satisfactory relationship of the degree of zinc deficiency symptoms, the plant composition, and the soil composition may have presented itself.

Table No. III A summary of the zinc found in the various parts of several sets of pineapple plants.

Plant Set No.	Sample Source	Growth Status	Soil Zinc	Zinc Deficiency Index	ZINC DETERMINED IN TISSUES SEGREGATED AS BELOW. ALL ANALYSES IN PARTS PER MILLION ON THE DRY BASIS.														
					"C" Leaves			"D" Leaves			"E" Leaves			"F" Leaves	Growing Points	Fruit Shell	Crowns Only	Slips & Suckers Only	Crowns Slips & Suckers
					basal	central	tips	basal	central	tips	basal	central	tips	entire					
p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.		
I	Libby Waipio C Oahu Pd. 137 (6 plants)	1 st ratoon, with fruit 3 mo. after blossoming	2.6	0	44	17	22	44	26	24	42	19	23		21			33	
II	T. Yonamine So. Halawa Oahu (8 plants)	Plant crop, 12 mo. slip planting (hold overs)	3.5	0	20	9	7	25	8	6	23	10	8	27	158				
III	K. Tokahara Aiea Hgts., Oahu (8 plants)	Plant crop, 10 mo. slip planting	1.7	0	16	6	4	16	9	12	24	13	10	26	144				
IV	Y. Yonashiro Aiea Hgts., Oahu (8 plants)	Plant crop, 16 mo. slip planting (hold overs)	0.8	1	20	8	4	20	11	7	26	13	9	22	96				
V	Libby Waipio C Oahu Pd. 137 (6 plants)	1 st ratoon, with fruits 3 mo. after blossoming	0.9	2	22	15	10	21	14	15	26	17	26		26			27	
VI	Trop. Frt. Ex., Red Hill, Oahu (6 plants)	Plant crop with fruit 3 mo. after blossoming	0.6	3	21	8	4	19	8	6	16	7	5		20	17	12		
VII	Trop. Frt. Ex., Red Hill, Oahu (6 plants)	1 st ratoon, suckers, no evidence of red-bud	3.9	3	21	10	5	21	11	6	16	13	10	21	81				
VIII	S. Tawata Kalahao, Kauai (3 plants)*	Plant crop, 12 mo. crown planting	0.6	4	7	5	8	8	5	8	13	6	8	12	23				
IX	S. Tawata Kalahao, Kauai (2 plants)*	Plant crop, 12 mo. crown planting	0.5	5	4	Combined "C" central-tips 8		Combined "D"- "E"- "F" basal 7			Combined "D"- "E"- "F" central and tips 6				6				

* Hilo Cayenne variety. All others were Smooth Cayenne.

Although the possibility of using the concentration of zinc in the growing points of the pineapple plant presents itself as a means of diagnosing zinc deficiency, this is hardly adaptable to practical agriculture.

These data indicate that the deficiency symptoms which are corrected by the spraying of zinc sulfate solutions on pineapple plants are associated with an insufficient concentration of the micro-nutrient in certain tissues of the plant. Although the "D" leaves are the ones that most readily show the symptoms of zinc deficiency, there is no relation between the concentration of zinc in these leaves and the degree of zinc deficiency.

From these limited data, however, it may be concluded that there exists a definite correlation of the available soil zinc, the zinc found in the growing point tissues, and the symptoms of zinc deficiency exhibited by the plants.

SUMMARY

This paper deals with a study of the determination, and concentration of zinc in pineapple plants and soils; consideration is given to: (1) the development of procedures for preparing solutions of plant materials, and soil extracts, for the determination of zinc by the polarographic method; (2) the symptoms of zinc deficiency exhibited by pineapple plants; and (3) the relationships of observed symptoms, the concentration of available soil zinc, and the concentration of zinc in the various tissues of normal and abnormal pineapple plants; and may be summarized as follows:

(A) A rapid, direct procedure for the polarographic determination of zinc in pineapple plants was developed. The essential features of this procedure are a wet combustion followed by the removal of phosphate by absorption with meta-titanic acid.

(B) A procedure for determining available zinc in soils is proposed.

(C) A relationship apparently exists between the degree of zinc deficiency exhibited by pineapple plants and the available zinc found in soils.

(D) A study of the distribution of zinc throughout pineapple plants revealed that the meristematic tissues contained the greatest concentrations of zinc. The growing points of zinc deficient pineapple plants were found to contain less zinc than normal or slightly zinc deficient plants.

(E) Apparently the abnormalities of pineapple plants cured by spraying with zinc sulfate are a direct result of the soils' inability to supply sufficient zinc to the plants.

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