

THE USE OF 0.1N HCl-EXTRACTABLE ZINC IN
ASSESSING AVAILABLE AND FIXED ZINC
IN HAWAIIAN SOILS

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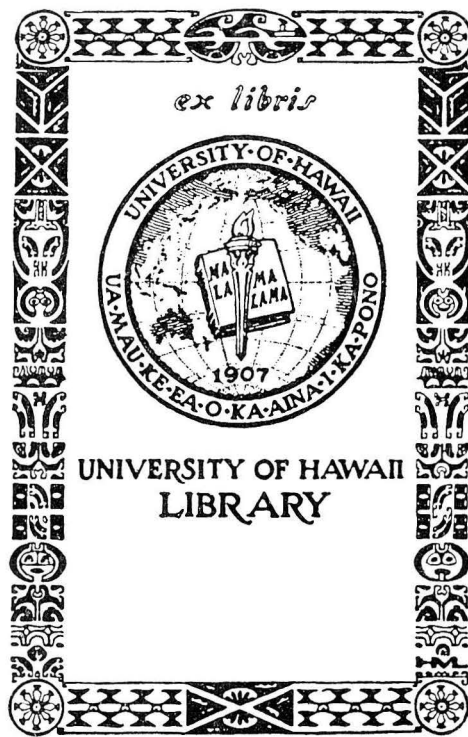


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INTRODUCTION

The essentiality of zinc to plants was reported in 1914, 1915, and 1919 and proved conclusively in 1926 and 1928(48). Since then zinc deficiency has been recognized in many areas, chiefly in Australia and in the United States. A map of the U.S. prepared by Beeson(3) shows extensive zinc deficiency areas in the Southeastern states, in Texas, California, Washington, as well as in many other states.

Common visual symptoms of zinc deficiency as reviewed by Thorne(48) and by Seatz and Jurinak(40) are: mottling, "little-leaf", rosetting, dieback, and the production of abnormal fruits in fruit trees; chlorosis and stunting in field crops; and "white top" in corn.

The history of zinc research and recent advances in this field are reviewed extensively by Thorne(48).

In Hawaii Lyman and Dean(30) reported zinc deficiency in pineapple manifested by blistering and mottling of leaves and by curvature of the "heart" leaves. This latter symptom, which has also been reported in Australia(1), is termed "crookneck". Shoji and Ota(44) reported chlorosis and stunting in coffee in several areas of Hawaii which could be corrected by foliar applications of zinc but not by soil applications. At the present time zinc research in Hawaii is conducted by the University of Hawaii Agricultural Experiment Station, the Pineapple Research Institute, and the Hawaii Sugar Planters' Association. Obviously, zinc deficiency is recognized as an important problem in Hawaiian soils.

This study was initiated to determine if, by correlation of zinc uptake by plants with acid-extractable soil zinc, the zinc available to plants can be reasonably estimated, and by fixation studies, if some insight could be gained on the causes of the apparent zinc fixation in Hawaiian soils.

The results of this study should determine whether or not acid-extractable zinc can be used to determine deficiency levels of zinc, and provide more information on the behavior of zinc in Hawaiian soils. This information could lead to further research on zinc availability to plants and fixation in soils.

REVIEW OF LITERATURE

A. Extractable and Available Zinc.

The use of neutral normal salt solutions for zinc extraction has been shown to be very unreliable. Jones et al.(23) found N $\text{NH}_4\text{OC}_2\text{H}_4$ (hereafter NH_4Ac) unsatisfactory for their work with Florida soils. Boawn(4), Brown(8), and Tucker and Kurtz(49) have also found N NH_4Ac to give unsatisfactory results.

At the present time, the dithizone-ammonium acetate extraction method of Shaw and Dean(41) appear to be the most satisfactory of available methods. Good results were obtained in the correlation of extractable zinc with zinc uptake and in the determination of a critical of extractable zinc(9, 31, 33, 41, 49, 50). By this method, the critical level of extractable zinc in soils, which is that level below which zinc deficiency in plants may be expected to occur, has been estimated to be about 0.5 and 0.55 in many soils(9, 41).

Another apparently suitable extractant is 0.1N HCl(4, 49, 50, 52). Wear and Sommer(58) found 0.1N HCl extractable zinc correlates well with the appearance of deficiency symptoms in corn. Using a 10:1 acid-soil ratio and shaking for one hour, they found a critical level of acid-extractable zinc to be between 0.90 and 1.20 ppm. Tucker and Kurtz(56) similarly recommended a 10:1 acid-soil ratio but found a 45-minute shaking time sufficient. Viets et.al.(50) reported similar total zinc contents in a deficient and a non-deficient soil. However, the latter soil contained more 0.1N HCl-extractable zinc. The work of Boawn et al.(7) upholds¹ the contention that acid-extractable zinc is an estimation of the available zinc in soils. By using several zinc compounds of differing solubilities in uptake studies with sorghum and beans, they found that the availability of zinc to these plants was greatest with those compounds most soluble in 0.1N HCl regardless of their solubility in water.

Both dithizone and 0.1N HCl have been shown to be good extractants although the latter does extract more zinc. The dithizone method is more universal in that it can be applied to all soils. The HCl method obviously cannot be applied to calcareous soils(49). However, the dithizone method is tedious and time consuming while the acid-extraction method is simpler and more rapid. Since most Hawaiian soils are acid and a rapid method is always desirable, 0.1N HCl was selected as the extractant in this study.

B. Zinc Fixation by Clay Minerals.

As mentioned earlier, the application of zinc to the soil was not successful in alleviating zinc deficiency symptoms in coffee in Hawaii(44). Similarly, Butler and Bray(10) found that Zn-EDTA applications to a silty loam produced no increase in zinc uptake in plants although the same treatment on a sandy soil did result in an increase in zinc uptake. They felt that the clays of the silty loam stripped the zinc from the chelated complex and rendered it unavailable to the plants. Lingle and Holmburg(28) and Boawn(7) found zinc from Zn-EDTA and from ZnO applications to be taken up readily but found that zinc from ZnSO₄ applications to be relatively unavailable to plants.

Water soluble and exchangeable zinc occurs in the soil in very small amounts. Hibbard(17) and Boawn(4) report that, after the addition of small amounts of zinc to soils, practically none was water soluble. Jones et al.(23) has found that ^{acid} water soluble zinc in sandy soils are readily leached and this is substantiated by Barrows et al.(2)

The failure of N NH₄Ac solution as an extractant as previously mentioned indicates that only small amounts of zinc exist as exchangeable ions in the soil(4, 49). Several workers have found that zinc apparently occupy "fixation" sites rather than exchangeable sites on clay minerals. Nelson and Melsted (35) found that the cation exchange capacity of soils and clays with fixed zinc was not different from that of the original untreated soils and clays. This indicates adsorption

or fixation of zinc on positions other than exchangeable. The work of DeMumbrum and Jackson(12) on zinc adsorption by soil colloids support this concept. Elgabaly(14), after working with several layered silicate clays, concludes that zinc can enter the crystal lattice of clays without ionic substitution or enter with substitution of ions of lower valence or enter with substitution of ions of equal valence. He has found evidence that zinc can replace magnesium in the crystal lattice of the clay minerals. This stand is supported by the fact that zinc and magnesium ions are of similar sizes. Furthermore, with infra-red absorption studies, DeMumbrum and Jackson (13) have shown that zinc reacts with the structural hydroxyl groups of the clay minerals and is thus integrated within the crystal lattice. Further still, Boawn(4) reports the identification by Ross of sauconite which is considered to be a saponite in which zinc has replaced magnesium. Saponite in turn is a montmorillonite in which magnesium occupies the octahedral positions.

The type of clay minerals in the soil is important in the fixation of zinc. It has been found that clays with high cation exchange capacities are better fixers than clays which have low cation exchange capacities and that magnesium clays and weathered clay minerals are good zinc fixers(2, 14).

From these evidences, it is apparent that the clay minerals in soils could influence zinc availability to some extent.

Clay mineral fixation of zinc was considered in this study because of the high clay content in Hawaiian soils. While many

of the soils in Hawaii are classified as silty clay, actually they are clays that are well aggregated and so give the appearance of a silty clay(45).

MATERIALS AND METHODS

A. Soils Used.

1. Akaka silty clay. This soil, a hydrol humic latosol, was formed on volcanic ash under an annual rainfall of 256 inches. The distribution of the rainfall is such that there is no pronounced dry season. This soil holds up to three times its weight in water and when dried, cannot be rewetted to its original state(25, 43, 45). Some of the properties of this soil are: pH, 5.2; cation exchange capacity, 80-100 m.e. per 100g soil(oven-dry basis) in its natural state and less than 40 m.e. when dehydrated; base saturation, less than 3% of the cation exchange capacity(25, 45). The clay fraction, which makes up practically all of this soil consist of: 30% allophane, 25-35% gibbsite, and 10-35% goethite(47). The B horizon was used in this study since there was a thin dehydrated layer on the surface of this soil. It was collected from a road-cut near Hilo, Hawaii.

2. Honuaulu clay loam. This soil, a humic latosol, was formed on a thin layer of ash under a rainfall of 65 inches per year which was not uniformly distributed(25,45). The pH of this soil is 5.6, its cation exchange capacity is 64.1, and its base saturation is 58%(25). The clay fraction of this soil is as follows: koalin, 15-20%; gibbsite, 15-20%; hematite, 20-

25%; goethite, 10-20%; and montmorillonite, 5-10%(47). This soil, which is the coffee soil of Kona, Hawaii, is very young and shallow; usually not more than 24 inches deep(45). The soil for this study was collected from a check plot of a zinc experiment field at the Kona station of the University of Hawaii Agricultural Experiment Station and is one of the soils that has shown zinc deficiency.

3. Wahiawa silty clay. This low humic latosol was formed on basaltic lavas under an annual rainfall of 40 inches, most of which falls in the fall and winter months; thus, there is a pronounced dry season of about six months. Some of the properties of this soil are as follows: pH, 5.1; cation exchange capacity, 17.8 m.e.; and base saturation, 53.4%(25,45). The composition of the clay fraction of this soil is: kaolin, 45-55%; hematite, 20-25%; allophane, 5-10%; and montmorillonite, 5-15%(47). This soil was collected from a macadamia nut orchard at the University of Hawaii Agricultural Experiment Station farm at Poamoho, Oahu.

4. Lualualei clay. This soil, a dark magnisium clay, is found in low-lying areas of poor drainage which recieve bases from rock weathering in the surrounding uplands. This creates an alkaline system which results in the formation of montmorillonitic clays(45). The cation exchange capacity of this soil is 51.7 m.e., its base saturation is 100%, chiefly calcium and magnisium(25). The pH of this soil is 7.42. The soil for this study was collected from a freshly plowed field in Lualualei Valley, Oahu, where the annual rainfall is 20

inches(25).

B. Analytical Methods and Procedures.

At the present time, there are several satisfactory methods for the analysis of micro-amounts of zinc which include bio-assay, spectrographic, polarographic, and colorimetric techniques(48). The most popular method appears to be the dithizone colorimetric method as modified by Cowling and Miller(11), Holmes(18), Wear and Sommer(52), and Shaw and Dean(41).

In 1954 Rush and Yoe(39) introduced a colorimetric method that utilizes zincon(2-carboxy-2'-hydroxy-5'-sulfoformylbenzene) as a reagent for determining zinc after interfering ions have been removed by the anion exchange resin method proposed by Kraus and Moore(26). This zincon-resin method was later applied to analysis of zinc in plants(21) and in soils (19,38).

Jackson and Brown(21) point out several advantages of the Rush and Yoe method over the older dithizone method. First of all, the zincon method is more simple and rapid. This saves time and lessens the chance for contamination or loss of zinc to occur during analysis. Secondly, the use of CCl_4 , a toxic compound the effect of which is cumulative, is not necessary in this new method as it is in the dithizone method; thus, a real hazard is avoided by the analyst doing hundreds of determinations.

A further advantage is that the maximum allowable concentration of zinc in the final solution(the solution to be analyzed on the colorimeter) is grater for the Rush and Yoe

method, 2.4ppm(39), than for the dithizone method, 1.4 ppm(11).

Although the zincon method of zinc analysis is not as sensitive as the dithizone method, it is still very satisfactory considering the variability of soil and plant samples. If a more accurate method of zinc analysis is required, the separation of zinc from interfering ions can still be done by the anion exchange resin method which has been found to be very efficient(21).

Because of the rapidity, convenience, accuracy, and safety of the Rush and Yoe method of zinc analysis, it was adopted for this study with modifications from the procedures of Jackson and Brown(21), Pratt and Bradford(38), and the Department of Plant Physiology of the University of Hawaii(53).

1. Separation of zinc from interfering ions.

a. Reagents:

Dowex I anion exchange resin, 1-x8 linkage, analytical grade, 50-100 mesh.

HCl solutions: 2N, N, 0.005N.

HCl solutions for purifying resin: 12N, 6N.

b. Apparatus:

Pyrex glass columns. In this study, funnels were used as columns. The dimensions were: overall length, 23 cm; inside diameter at top, 10cm; stem length, 15 cm; inside diameter of stem, 5mm.

c. Preparation of the resin columns.

A wad of pyrex glass wool is placed into the bottom of the stem of the funnel. Water is added into the

funnel and, before all the water has passed through, enough resin suspended in water is slurried into the funnel to fill the stem. In this way the resin settles into the stem of the funnel without any air bubbles forming. Should any air bubbles form, no solution or water will pass through the column. When this happens, water is added into the column and a stirring rod of small diameter is used to stir up the resin and thus release the air bubbles.

By tightening the wad of glass wool in the stem the stem, the rate of flow through the column can be adjusted to flow at a rate of 15 ml per hour. Too rapid passage may result in incomplete separation of ions.

To purify the column, 10 ml of 12N, 6N, and 2N HCl and 50 ml of zinc-free water are passed through successfully in that order.

A short length of zinc-free plastic tubing is attached to the bottom end of the column so that it can be closed off with a pinchcock when not in use. The resin is kept wet at all times to prevent the formation of air bubbles in the column.

d. Separation procedure.

To separate zinc from the interfering ions, the unknown solution is ^{made} ~~made~~ 2N with HCl and passed through the column which has been pre-treated with 50 ml of distilled water and with 10 ml of 2N HCl.

After the unknown solution, about 60-100ml, has passed

through, 50 ml N HCl is passed through to wash out what interfering ions that remain in the column. Then 10 ml of N KCl is used to wash out the excess acid. All washings are discarded. Then a 100 ml volumetric flask is placed under the column and 50 ml of 0.005N HCl is passed through to extract the zinc. This eluate is then analyzed by the zincon method.

Although this separation procedure is slow, the man-hours needed is small. By setting up as many columns as desired, many determinations can be run at the same time and while the various solutions are passing through the columns, the analyst is free to do other chores.

2. The zincon method of zinc analysis.

a. Reagents:

Zincon

Boric acid

Ammonium hydroxide, concentrated

Sodium hydroxide

b. Preparation of the buffer solution.

Twenty grams of boric acid and 50 ml of concentrated ammonium hydroxide are mixed with enough water to make a liter of solution. The solution is then adjusted to a pH of 9.5.

c. Preparation of the zincon solution.

Dissolve 0.30 g zincon in 2 ml 2N NaOH. Dilute to 250 ml with water. This solution is good for about four days.

d. Procedure for zinc analysis.

After the zinc has been separated from the interfering ions, extracted from the resin column, and collected in a 100 ml volumetric flask, 10 ml of buffer and 10 ml of the zincon solution are added, and the whole made to volume. After standing for 25 minutes, the light transmission of the solution is read on a colorimeter at 620 m-microns wavelength. This is then compared to a standard curve made with zinc solutions of 0, 0.2, 0.4, 1.0, 1.5, and 2.0 ppm concentrations.

A Beckman Model DU colorimeter was used in this study.

3. Test of the Rush and Yoe Method of Zinc Analysis.

There was some concern as to whether or not water distilled only once was suitable for zinc analysis. Generally, de-ionized distilled water, re-distilled water, or even triple-distilled water is recommended for minor element analysis. However, because of the very low mineral content of the water available in the Honolulu area, it was felt that distilled water might be satisfactory. Once-distilled water has been used satisfactorily at the Departments of Plant Physiology and of Agronomy and Soil Science at the University of Hawaii.

To test the Rush and Yoe method using once-distilled water, several standard samples of 20, 50, 100, 150, and 200 ug of zinc were run through the analytical procedure. Except at the 20 ug level, which is at the 0.02 ppm level

on the standard curve, all results were satisfactory with not more than 4% error. Furthermore, two 100ug samples were deliberately contaminated with arbitrary amounts of CaCO_3 , MgCl_2 , BaCl_2 , FeSO_4 , and CuSO_4 , and the zinc recovery of these were satisfactory. Therefore, this method was deemed satisfactory for this work provided the 0.2 ppm part of the standard curve is avoided.

4. Preparation of plant material for zinc analysis.

A one or two gram sample of dried, ground(20 mesh) plant material is weighed into a 30 ml porcelain crucible and then ashed in a muffle furnace overnight at 400-450 degrees C. After the ashed sample has cooled, it is treated with an excess of N HNO_3 , evaporated to dryness, and then re-ashed at the same temperature for 10 minutes or more. The ash is then cooled and taken up with 2N HCl .

The solution is heated and then filtered through an S and S #589 white rebbon filter paper using 2N HCl to wash the residue. The filtrate is then analyzed for zinc by the Rush and Yoe method.

5. Preparation of the soil extracts for analysis.

The soil sample, usually 20 g(oven-dry basis), is placed in a 500ml Erlenmeyer flask and 10 ml of 0.1N HCl is added for each g of soil. The flask is stoppered and shaken for an hour on a mechanical shaker. The supernatent liquid is poured then into a 250 ml centrifuge tube and is centrifuged at 1500 rpm for as long as is necessary to settle the soil particles. An aliquot of the clear extract is taken,

filtered to remove floating organic matter, evaporated to dryness on a hot plate, digested with 10 ml of N HNO₃, and re-evaporated to dryness. The residue is taken up with 2N HCl and the solution is filtered through an S and S #589 white ribbon filter paper to remove insoluble substances. The filtrate is then ready to be analyzed.

C. Experimental Procedure.

1. Experiment I: Pot Study.

Ten pound samples(field-weight) of each soil except the Akaka silty clay were placed in plastic pots. Because of the low bulk density of the Akaka soil, only 8 lb was necessary to fill each pot.

Each pot was fertilized according to the recommendations of Jacob and von Uexkull(22) and limed to a pH between 5.5 and 7.0 as suggested by Foster and Matsusaka(15). The fertilizer and liming rates for these soils were: 147 lb K₂HPO₄ and 100 lb NH₂SO₄ per acre to all soils; 10 tons lime as CaCO₃ per acre to the Akaka silty clay, 4.0 tons per acre to the Honuaulu clay loam, and 1.5 tons per acre to the Wahiawa silty clay; 3 lb MgSO₄ per acre to the Akaka, Honuaulu, and Wahiawa soils. No lime nor any MgSO₄ was applied to the Lualualei clay since this is an alkaline soil with high contents of calcium and magnesium.

Zinc sulfate was applied on each soil in four replicates at rates of 0, 25, 50, 100, and 200 lb per acre based on one acre being equal to 2.5 million pounds of soil.

Because a great amount of relatively zinc-free water

would be necessary to water these pots, this experiment was conducted out of doors to take advantage of the rainfall. The rainfall in February and March on the University of Hawaii Honolulu campus is about five inches per month(6). By conducting this test out of doors the use of distilled water is avoided and use of tap water, which contains 0.09 ppm zinc, was kept to a minimum.

The fertilization and liming were done as surface applications in anticipation of the high rainfall which was expected to leach much of the materials through the pot.

Soybeans(Glycine soja) was selected as the test plants since it was said to be sensitive to zinc deficiency(50). The variety Bonsei was supplied by Dr. D.C. McGuire of the Department of Horticulture and was recommended because of the non-sensitivity of this variety to photoperiodism.

Five seeds were planted to each pot. After the plants were established, the pots were thinned to two plants per pot except for the pots containing the Lualualei clay. Only one plant was allowed for these pots because of the expected vigor of the plants growing on this soil. After the plants flowered, they were harvested, rinsed first with tap water then with distilled water. The plants were then dried overnight in a draft oven at 70 degrees C. The plants were then weighed, and then ground up with a Wiley mill fitted with a 20 mesh seive. Before weighing for analysis, each sample of plant material was re-dried.

After harvest, the soil in each pot was thoroughly mixed

and, except for the Akaka and Lualualei soils, passed through an 18 mesh stainless steel screen. Because of the stickiness of the Akaka and Lualualei soils, sieving of these soils was impossible except when these soils are dried. However, it was felt that drying would affect the extractable zinc contents so therefore no sieving was done. Furthermore, most of the material in these soils was of the 18 mesh size or smaller. Care was taken to remove rocks and other large materials.

The pH of the soils in each pot was determined with a Beckman Model G pH meter on a 1:1 soil-water paste.

The plant samples were analyzed by the stated procedure.

2. Experiment II: Fixation study.

An amount of $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ equivalent to 100 lb per acre was added to 20 g of soil(oven-dry basis) that had been passed through an 18 mesh sieve and put into a 500 ml Erlenmeyer flask. In this case, the Lualualei clay was dry enough to pass through the sieve so only the Akaka silty clay was unseived. One hundred lb of $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ per acre is equivalent to 230 ug zinc per 20 g soil or 11.5 ppm zinc.

The zinc treated soils were allowed to stand for periods of 1, 7, 14, and 28 days. During this time the soils were kept moist with distilled water. After the standing time the zinc was extracted with 0.1N HCl and the amount of zinc fixed was calculated. This experiment was replicated twice.

RESULTS AND DISCUSSION

The results of this study are tabled in the appendix.

Statistical tests were not applied to the data obtained in these experiments since, upon inspection, the variability of the results in most cases indicate that such tests were not warranted.

A. Experiment I: Pot Study.

The soils used in these studies all apparently supplied enough zinc to the soybean plants growing in them to maintain healthy growth. The plants showed no zinc deficiency symptoms nor any response to zinc applications. Moreover, their zinc content was high when compared to the 30 ppm encountered in healthy soybeans by Nelson(36). Correlation between zinc uptake and acid-extractable soil zinc was absent.

The 28.3 ppm initial acid extractable zinc content found for the Akaka silty clay(table 5a) is surprisingly high considering the extremely low base saturation of that soil. Highly base depleted, this soil would presumably also be depleted in most of the minor elements. Unless the land from which this soil was taken was fertilized or contaminated in some other way with zinc, the presence of this high zinc content was unexplainable.

Liming the Akaka silty clay greatly reduced the extractable zinc content(table 1). This is in accordance with the generally well established fact that an increase in soil pH results in a decrease in extractable and available zinc(27,31,37,42, 51). Jurinak and Thorne(24) have shown calcium zincates to be very insoluble. Several workers have reported the insolubility of zinc phosphates in neutral and alkaline systems(1). Hence,

pH might account for the reduced amounts of extractable zinc upon liming.

It should be pointed out however, that 0.1N HCl is not recommended for calcareous soil and that the limed Akaka soil may have acted as a calcareous soil in neutralizing the extractant. This also could account for the lower extractable zinc found in the limed soil.

The Honuaulu clay loam also displayed a high initial extractable zinc content(table 5a). As mentioned before, this soil has developed zinc deficiency in coffee. No deficiency symptoms appeared on any of the plants grown on this soil in this experiment. Apparently the section of the field from which this soil was collected was well supplied with zinc.

Liming this soil did result in lower amounts of extractable zinc(table 2). However, there was no increase in pH. Apparently the lime applied was not sufficient to overcome the buffering capacity of this soil. Again, the reduction in extractable zinc due to liming may be the result of neutralization of the extracting acid.

The plants growing in the Akaka silty clay were all chlorotic. However, this could not be attributed to zinc deficiency since the chlorosis was universal for all the plants growing on this soil regardless of zinc treatment. The deficiency in this soil is probably that of one or more of the major elements. D.L. King(personnal communication) in working with this soil has reported chlorotic Sudan grass despite repeated applications of major and minor elements. Dehydration

of this soil, which results in an irreversable change in its minerology, resulted in healthy plant growth. Obviously, the clay minerals play an important part in the availability of at least some of the plant nutrients.

The plants grown on the Wahiawa silty clay all displayed tiny rust-colored spots on the leaves. This symptoms was peculiar to the plants growing in this soil and appeared on all the plants growing in this soil. Therefore, the plants were believed to be influenced by some edaphic factor. Since the plants were otherwise normal and since the symptoms were noticed near maturity of the plants, no effort was made to determine the cause of the observed symptoms nor to correct it.

The plants on the Lualualei clay and the Honuaulu clay loam showed no visible symptoms of abnormal growth. However, there was an apparent negative correlation between zinc uptake and dry weight yield in the Lualualei clay, but the validity of this is questionable since theoretically this soil would be the least likely of those studied to display zinc toxicity. Being an alkaline soil of 2:1 clay content, zinc would be least available in it than in any of the other soils. Lott(29) has shown that severity of zinc toxicity is reduced with increases in pH until a pH of 5.7 is achieved beyond which no toxicity could be induced even with the application of 4000 ppm zinc. Gall and Barnette(16) have shown liming to increase the level of zinc concentration in soils at which toxicity to corn and cowpeas occur. Despite high applications of zinc, Speer et al. (46) found no toxicity on a Houston black clay, a soil

similar to the Lualualei clay. Y. Kanehiro of the Department of Agronomy and Soil Science at the University of Hawaii (personnal communication), working with the Lualualei clay, has shown no zinc toxicity with the application of 100 lb $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ per acre. Furthermore, in this study, the plants in all the check pots and in three replicates of the 25 lb ZnSO_4 per acre treatment, among others (table 4), were grown later in drier weather. This was necessitated by heavy rains at the initial planting which left the Lualualei clay water-logged and even with standing water. Many of the soybean seeds were rotted in this excessive moisture. Those that were not were allowed to grow. The others were replanted as soon as the soils were dry enough. Thus, the weather is believed responsible for the apparent negative correlation between zinc uptake and plant yield.

The application of ZnSO_4 to the soils resulted in no apparent significant increase in zinc uptake by soybean plants although there apparently was increases in acid-extractable zinc. The lack of increase in zinc uptake might have been the result of zinc fixation by the soil or because of the inability of plants to absorb the available zinc.

Fixation of zinc by clay minerals as reviewed earlier has been substantiated by much evidence. Fixation obviously occur in Hawaiian soils as is evidenced by the failure of soil applications of zinc to correct deficiency symptoms(44). However, if fixation is responsible for the non-increase in zinc uptake with treatment, then the acid-extractable zinc which

does increase, must contain zinc that is unavailable to plants. If this is true, then the acid-extractable zinc is not an assessment of the available zinc in Hawaiian soils. These findings coincide with that of Leyden and Toth(27) who reported that yield and zinc uptake was not affected by $ZnSO_4$ application and nor did yield and uptake correlate with acid-extractable zinc. They also found that zinc fixation against 0.1N HCl is apparently associated with the nature of the clay minerals in the soil and with the cation exchange capacity of the soil. They concluded that 0.1N HCl-extractable zinc was not an indication of the available zinc in the soil. Nelson and others(34) also reports unsatisfactory results with HCl extraction. They proposed a method in which the soil is titrated to a specified pH and then extracted for zinc with 0.1N HCl. In this way, they found satisfactory results on both acid and alkaline soils.

Another factor to consider in the non-correlation of acid-extractable zinc and zinc uptake is the excessive moisture experienced during the growing period which may have influenced the uptake of zinc by the plants. A lack of aeration, which results in a repression of water and elemental absorption by plant roots(5), could conceivably account for the lack of increase in zinc uptake with increases in application. If this has occurred in this experiment, then zinc in available forms in the soil, which may be acid-extractable, was not taken up because of the lack of aeration rather than the lack of available zinc in the soil. It is not known how significant was the

role of aeration in this experiment.

Also to be considered in the non-correlation of zinc uptake and extractable zinc is the fact that all the soils supplied enough zinc to the plants. Hence, to determine whether or not zinc is available, it must be assumed that soybeans will absorb luxury amounts of available zinc. If the plants cannot, then the lack of increase in uptake with treatment would be accounted for. However, since some of the plants in the Lualualei soil(table4) had zinc contents of over 70 ppm, the assumption that soybean plants will absorb luxury amounts of zinc if it is available is believed valid.

B. Experiment II: Fixation Study.

The results of the fixation study may indicate that the soils fix zinc according to the properties of their clay minerals.

The Akaka silty clay, with practically no layered aluminosilicate clays, apparently fixes very little zinc if any despite its very high cation exchange capacity(table 5b). This indicates that what zinc is adsorbed can readily be extracted with 0.1N HCl.

The Honuaulu clay loam and the Wahiawa silty clay fixed zinc immediately with no further increase with standing time although the latter fixes very little zinc in one day. This is probably because of the low cation exchange capacity of that soil and the resultant lack of contact between the zinc and the exchange sites of the clay particles.

The Lualualei clay appeared to fix zinc in increasing amounts with increasing standing time. This might be attributed

to the greater specific area of the montmorillonitic clays of this soil. With the increasing standing time, presumably zinc ions could work its way into exchange sites between the expanded layers. However, whether or not zinc is mobile enough in this alkaline soil is subject to question since, as pointed out before, zinc is not soluble in alkaline soils and would be expected to precipitate out of solution.

The amount of zinc fixed may be a reflection of the calcium saturation of the soil. Nelson and Melsted(35) have found zinc to become fixed in a calcium saturated clay but not in a hydrogen-ion saturated one. The calcium saturation of the soils studied are: Lualualei clay, 75.9%; Honuaulu clay loam, 36.3%; Wahiawa silty clay, 31.2%; and Akaka silty clay, 1.2%(25). When this is compared to the fixation results in table 5b, an apparent negative correlation appears between the amount of zinc fixed and the calcium saturation of the soil.

Two things of note resulted from the fixation tests which may deserve further study. First, there was an indication that the Buffering capacity of the soils may determine to some extent the feasibility of using HCl for zinc extraction. Table 5a shows that the precision or reproduceability obtained was best in the Wahiawa silty clay which has a low buffering capacity, intermediate in the Lualualei and Honuaulu soils which have intermediate buffering capacities, and poor in the Akaka silty clay which has a high buffering capacity(32). It might be noted that where 0.1N HCl has been successfully employed as an extracting agent(4,49,50, 52), sandy soils

were used since deficiency occur principally on these soils. The buffering capacity of these soils must obviously be low.

Secondly, the apparent lack of fixation in the Akaka silty clay indicates that the amorphous material which make up most of this soil(45) does not fix zinc. The other soils, all of which fix some zinc, have appreciable contents of layered alumino-silicate clays. That these clays fix zinc is in accordance with the findings of other workers as mentioned previously.

The evidence is far from conclusive and more study is required to formulate definite concepts.

SUMMARY AND CONCLUSIONS

This study was conducted to determine if 0.1N HCl-extractable zinc can be used to assess the available zinc status and to gain more information on zinc fixation in Hawaiian soils.

A pot study was set up using four Hawaiian soils: the Akaka silty clay, the Honuauulu clay loam, the Wahiawa silty clay, and the lualualei clay. After fertilization and liming, $ZnSO_4$ at rates of 0, 25, 50, 100, and 200 lb per acre was applied to each soil in four replicates. Soybeans were planted to these pots and after harvesting, the dry weight yield was recorded. The soils were then analyzed for acid-extractable zinc, and the plant tops for zinc.

The results showed no increase in zinc uptake with applications of $ZnSO_4$. Hence, there was no correlation between acid-extractable zinc and zinc uptake.

Using the same four soils as in the preceding experiment,

a fixation study was conducted by applying $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ to 20 g soil samples (oven-dry basis) at a rate of 100 lb per acre. The zinc was then extracted with 0.1N HCl, analyzed, and the amount fixed calculated.

Except for the Akaka silty clay, each of the soils used in this study fixed zinc against 0.1N HCl extraction. This indicates that the amorphous constituents of the Akaka soil is incapable of fixing zinc and supports the contention that zinc is fixed by crystalline alumino-silicates clays. However this also could mean that Zn is not fixed in a hydrogen saturated soil.

Also resulting from this experiment was an indication that acid-extractable zinc is influenced by the buffering capacity of the soil since the precision was best in the soil with the lowest buffering capacity, intermediateⁱⁿ the soils with intermediate buffering capacities, and poorest in the soil with the highest buffering capacity.

It is concluded from this study that 0.1N HCl, which has been used successfully in measuring available zinc in sandy soils, is not satisfactory for Hawaiian soils which are mostly clay. However, this conclusion is valid only if the high rainfall experienced during this study and the adequate initial amounts of zinc in the soil did not restrict uptake by the plants used in this investigation,

APPENDIX

Table 1: (Experiment I) Effect of ZnSO_4 on Soybean Yield and Zinc Content, and on the 0.1N HCl-Extractable Soil Zinc When Applied to the Akaka silty clay.

ZnSO_4 /Acre lb	Repli- cate	pH of Soil at Harvest	Dry- Weight Yield g	Zn in Plant Tops ppm	Extractable Zn in the Soil ppm
0	1	7.0	3.67	29	1.65
	2	7.1	2.11	29	3.94
	3	7.5	3.28	24	5.53
	4	7.0	3.48	22	3.57
	avg		3.13	26	3.67
25	1	7.1	3.47	46	8.52
	2	7.1	2.76	48	6.88
	3	7.3	2.71	46	11.27
	4	7.3	3.48	42	10.07
	avg		3.10	46	9.18
50	1	7.1	3.45	46	20.85
	2	7.0	3.82	22	4.25
	3	7.0	2.78	53	18.10
	4	7.3	3.11	32	5.64
	avg		3.29	38	12.21
100	1	7.0	2.61	24	5.80
	2	7.3	3.74	41	4.60
	3	7.2	1.91	47	7.76
	4	7.1	2.48	42	4.27
	avg		2.68	38	5.61
200	1	7.1	3.27	22	5.51
	2	7.0	2.85	32	3.38
	3	7.0	2.95	28	4.30
	4	7.2	3.20	30	7.44
	avg		3.06	28	5.16

Table 2: (Experiment I) Effect of ZnSO_4 on Soybean Yield and Zinc Content, and on the 0.1N HCl-Extractable Soil Zinc When Applied to the Honuaulu clay loam.

ZnSO_4 /Acre lb	Repli- cate	pH of Soil at Harvest	Dry- Weight Yield g	Zn in Plant Tops ppm	Extractable Zn in the Soil ppm
0	1	5.5	4.22	40	8.13
	2	5.4	2.48	46	5.69
	3	5.4	3.59	36	5.17
	4	5.4	3.17	50	5.78
	avg		3.36	43	6.19
25	1	5.5	3.72	46	7.15
	2	5.5	3.26	43	6.93
	3	5.4	2.94	46	8.51
	4	5.4	3.72	42	7.27
	avg		3.41	44	7.46
50	1	5.2	3.03	49	8.97
	2	5.3	3.53	40	8.58
	3	5.2	4.48	39	13.66
	4	5.3	2.92	42	13.58
	avg		3.49	42	11.20
100	1	5.3	3.55	48	9.90
	2	5.2	3.87	38	8.50
	3	5.3	3.35	47	9.45
	4	5.4	4.89	41	13.94
	avg		3.91	44	10.45
200	1	5.5	3.45	43	14.56
	2	5.4	3.05	51	17.02
	3	5.5	3.34	46	13.76
	4	5.5	4.29	42	14.19
	avg		3.53	45	14.88

Table 3: (Experiment I) Effect of ZnSO_4 on Soybean Yield and Zinc Content, and on the 0.1N HCl-Extractable Soil Zinc When Applied to the Wahiawa silty clay.

ZnSO_4 /Acre lb	Repli- cate	pH of Soil Harvest	Dry- Weight Yield g	Zn in Plant Tops ppm	Extractable Zn in Soil ppm
0	1	6.1	1.80	34	3.54
	2 ¹	5.9	4.51	36	2.94
	3	6.2	5.31	36	3.35
	4	6.2	2.57	42	2.78
	avg		3.55	37	3.15
25	1	6.2	2.62	47	4.89
	2	6.1	3.03	35	3.68
	3 ¹	6.0	4.38	40	3.35
	4	6.0	2.49	46	3.09
	avg		3.12	42	3.75
50	1	6.0	2.57	36	3.70
	2	6.1	2.65	46	3.44
	3	6.1	2.19	35	3.79
	4	6.1	3.33	29	3.61
	avg		2.68	37	3.64
100	1	6.1	2.26	35	6.68
	2	6.0	1.73	41	3.75
	3	6.0	2.63	33	4.81
	4	6.1	4.45	34	5.58
	avg		2.76	36	5.20
200	1	6.0	2.13	40	4.60
	2	6.2	2.22	41	3.86
	3	6.2	4.01	36	5.47
	4	6.2	3.98	46	4.92
	avg		3.08	41	4.71

¹ Planted later than the other pots because the first planting failed.

Table 4: (Experiment I) Effect of ZnSO_4 on Soybean Yield and Zinc Content, and on the 0.1N HCl-Extractable Soil Zinc When Applied to the Lualualei clay.

ZnSO_4 /Acre	Repli- cate	pH of Soil at Harvest	Dry- Weight Yield g	Zn in Plant Tops ppm	Extractable Zn in the Soil ppm
0	1 ¹	7.5	10.93	42	3.87
	2 ¹	7.3	8.44	43	3.03
	3 ¹	7.6	7.00	47	3.86
	4 ¹	7.5	10.18	37	3.88
	avg		9.14	42	3.66
25	1 ¹	7.8	4.36	70	5.73
	2 ¹	7.6	8.02	28	3.11
	3	7.5	7.45	44	3.98
	4 ¹	7.7	5.27	56	4.99
	avg		6.28	48	4.45
50	1 ¹	7.4	3.43	69	4.92
	2 ¹	7.8	3.92	74	4.73
	3	7.3	3.79	32	4.78
	4	7.4	4.76	23	3.90
	avg		3.98	48	4.58
100	1 ¹	7.6	3.52	76	6.04
	2 ¹	7.7	3.19	71	5.84
	3	7.8	1.62	42	4.00
	4 ¹	7.4	7.00	46	4.86
	avg		3.83	59	5.18
200	1	7.4	2.91	43	8.50
	2 ¹	7.4	3.52	75	8.97
	3	7.5	3.12	35	4.89
	4 ¹	7.6	7.65	46	5.86
	avg		4.30	50	7.06

¹ Planted later than the other pots because the first planting failed.

Results of Experiment II: Fixation Study

Table 5a: Initial 0.1N HCl-Extractable Zinc in the Soils Studied Expressed in ppm.

Replicate	Akaka	Monuaulu	Lualualei	Wahiawa
1	27.9	8.7	3.5	1.8
2	28.7	8.4	3.5	1.4
avg	28.3	8.6	3.5	1.6

Table 5b: Zinc Fixed on Standing with 100 lb ZnSO₄·H₂O/Acre (11.5 ppm Zinc) by the Soils Studied Expressed in ppm.

Time Days	Repl- cate	Akaka	Monuaulu	Lualualei	Wahiawa
1	1	0.2	4.0	3.6	0.4
	2	-3.4	4.0	4.6	0.8
	avg	-1.6	4.0	4.1	0.6
7	1	3.4	3.8	3.6	3.2
	2	2.3	4.6	5.4	3.4
	avg	3.1	4.2	4.5	3.3
14	1	2.2	3.1	-1	2.8
	2	-2.0	4.1	4.6	2.6
	avg	0.1	3.6	4.6	2.7
28	1	-7.0	5.8	5.5	3.4
	2	-2.0	3.4	5.3	3.2
	avg	-4.5	4.6	5.4	3.3

* 0.8 was obtained and rejected because of its apparent erraticness.

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