EVALUATION OF ZINC STATUS OF SEVERAL ECUADORIAN SOILS

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY
FERNANDO PACIFICO TORRES

1974

ABSTRACT

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SEVERAL ECUADORIAN SOILS

By

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A short-term greenhouse cropping procedure was developed to evaluate the zinc status of soils. In this procedure, corn plants are grown in sand culture in such a way that the actively-growing roots can be placed in contact with a small volume of soil (100 grams) for two weeks. This results in an exhaustive removal of available zinc from the soil, and the zinc content of the corn plants gives a measure of the zinc status of the soil. In each cropping experiment, a standard soil known to be deficient in zinc is included to provide a means for comparison.

By using this technique to evaluate the zinc status of 10 Ecuadorian soils, it was determined that 3 of the 10 soils were possibly deficient in zinc. Therefore,

field experiments are advisable at these locations to evaluate possible zinc responses.

Total zinc content of the soils was not related to the level of available zinc in the soils. However, linear correlation analyses indicated that any one of three methods of extracting available soil zinc (0.1 N HCl, EDTA, and DTPA) could be used to evaluate the zinc status of the soils. The results suggested that DTPA would probably be the most satisfactory extractant because of a higher level of correlation and because the use of this extractant can be more easily adapted to routine laboratory procedures.

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by

Fernando Pacifico Torres

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Crop and Soil Sciences

1974

<u>With all my love</u>

This thesis is dedicated to my wife, Carmen, and to my lovely children, who bolstered my spirit at times when I needed it.

ACKNOWLEDGMENTS

During the course of my research I have been fortunate to receive the generous and able assistance of many persons. I am pleased to acknowledge here my indebtedness to them and to recognize those that provided special help.

Sincere gratitude is acknowledged to Dr. Eugene C. Doll, my major professor and chairman of my thesis committee, for his immeasurable aid, unfailing courtesy, and expert guidance in directing my thesis and making my work both pleasant and rewarding.

General acknowledgement is due to INIAP (Instituto Nacional de Investigaciones Agropecuarias) for its financial support and stimulation to pursue a higher degree of education.

Appreciation is expressed to Dr. Bernard Knezek for his enthusiastic assistance. Thanks are also extended to Dr. Donald R. Christenson, Dr. Lynn S. Robertson, Dr. John Shickluna and Dr. Robert Rupple, members of my thesis committee.

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INTRODUCTION

Many of the soils in the Andean mountain valleys of Ecuador require relatively heavy applications of phosphorus fertilizer if maximum crop yields are to be obtained. A number of field experiments with corn have been conducted in recent years in which heavy broadcast applications of phosphorus were applied. In some instances, corn yields were reduced, or initial corn growth was retarded on plants which received high rates of phosphorus. On some plants, symptoms developed which were characteristic of zinc deficiency. As far as the writer knows, no studies related to the zinc status of Ecuadorian soils has been conducted.

Therefore, samples from Ecuador were obtained from typical agricultural soils of the Ecuadorian Andean valleys for further studies at Michigan State University. The study reported here was conducted to (1) develop a quick greenhouse procedure to determine the relative zinc status of different soils and (2) using this procedure, evaluate the zinc status of the Ecuadorian soils. This method could then be used to evaluate the zinc levels in soils from many different locations to determine where more intensive field experiments would be warranted.

REVIEW OF LITERATURE

Most soils contain micronutrients in sufficient quantities to sustain normal plant growth. On the other hand, zinc (Zn) deficiency in agricultural crops is one of the most common micronutrient deficiencies, and can develop in some soils due to crop removal, leaching, chemical fixation, erosion, or an initial lack of primary minerals which serve as sources of Zn.

Leeper (1952) listed several factors which affect the availability of micronutrients to plants. Depending on the ionic species, availability is influenced by low total content, low exchangeable content, organic matter and calcite complexes, anion precipitation, aging and recrystallization, and competition among species.

Role of Zinc in Plants

Literature on the critical concentration of Zn for normal growth of plants indicates considerable variation among species and varieties. Ellis (1965) reported that in Michigan it has been shown that the Saginaw variety of navy bean may yield well in the same soil where the Sanilac variety develops marked Zn deficiency; with adequate Zn fertilizer, the highest yields can be obtained with the Sanilac variety.

The following tentative classification of crops was given by Viets _et al_. (1954) based upon sensitivity to Zn deficiency:

Highly sensitive: beans, soybeans, corn, hops, grapes, lima beans, flax and castor beans.

Moderately sensitive: potatoes, tomatoes, onions, alfalfa, grain sorghum, sudan grass, sugarbeets and red clover.

Insensitive: peppermint, oats, wheat, barley, rye, peas, asparagus, mustard, carrots, safflower, and grasses.

Boehle and Lindsay (1969) listed cotton and fruit crops, especially citrus and peach, as good indicators of Zn deficiency.

Salisburg and Ross (1969) and Boehle and Lindsay (1969) stated that Zn is needed for the proper utilization of carbon in plants. It is needed for protein metabolism and forms part of the enzyme systems which regulate plant growth. It is a constituent of the enzyme carbonic anhydrase, a catalyst which breaks down carbonic acid. Zinc is necessary for the formation of trytophan, a precursor of indoleacetic acid (IAA). IAA is the most prevalent hormone in plants and Zn deficient plants have greatly reduced auxin activity. Auxin content is important because changes in water content of plants are directly related to changes in the auxin content.

As cited by Price et al. (1972), B. L. Vallee first recognized the role of Zn as an essential component of a variety of dehydrogenases, proteinases, and peptidases. Epstein (1972) noted that Zn is the metal component of a number of metalloenzymes, including several dehydrogenases, among them alcohol dehydrogenase and lactic dehydrogenase.

Salisburg and Ross (1969) suggested that Zn is related to chlorophyll formation because the symptoms of Zn deficiency in several fruit trees is an interveinal chloroses in the leaves.

Salisburg and Ross (1969), Lindsay (1972), and Epstein (1972) all state that while all nutrient deficiencies reduce plant growth, a lack of Zn frequently reduces growth so dramatically that terms like "little leaf," "rosette," "mottle leaf" or "yellows" have been applied to the condition.

Zinc in Soils

Swaine (1955) reported that the total Zn content of soils varies from 10 to 300 ppm and that only part of this Zn is available for plant growth.

The incidence of Zn deficiency in the United States has increased since Beeson (1945) mapped those states where deficiencies occurred. Berger (1962), as cited by Kubota and Allaway (1972), Lindsay (1972) and Cox (1973) reported that Zn deficiencies had occurred in 30 states, and Viets in 1966 reported that at least two additional states could be added to this list because he had observed symptoms of deficiency in crops in areas where these symptoms had not been evident 2 or 3 years earlier.

As cited by Lindsay (1972), Ryan _et al_. in 1967 reported that low Zn levels occur in 10 of 15 European countries and in Israel. As the search continues, the areas of deficiency throughout the world are expected to expand.

Hibbard (1940), Mitchell (1964), Lindsay (1972) and others agree that in general, the A_1 horizon contains the greatest quantity of Zn because of the high organic matter content.

Krauskopf (1972) pointed out that the common minerals which are the principal sources of Zn in soils are (1) sulfide as sphalerite (ZnS), (2) carbonate as smithsonite ($ZnCO_3$), (3) silicate as hemimorphite (Zn + $(OH)_2Si_2\ O_7 \cdot H_2O$). Zinc occurs dominantly in both silicates and sulfides. The carbonate and sulfide of Zn are slightly soluble. He also reported that Zn is found in a clay mineral (sauconite) in which it is an essential constituent, but the pure Zn mineral is rare. Zinc also occurs in sedimentary rocks as disseminated grains of sphalerite, often accompanied by galena, especially in carbonate rocks. Lindsay (1972) pointed out that sphalerite can form under reducing conditions where H_2S is produced.

According to Lindsay (1972) Zn deficiencies are frequently found in areas where the surface soil has been removed. He added that farmers have found that liberal application of manure and other organic materials are often effective in correcting Zn deficiencies. On the other hand, Zn deficiencies are often noted on old corral sites that are high in organic matter. This apparent contradictory behavior is explained because organic matter can interact with Zn in two ways. First, soluble Zn can be mineralized and made available to plants. Second, Zn can be incorporated into organic constituents that are immobile in soils and fix Zn in a form from which it is not readily released.

Factors Affecting Zinc Availability

There are several factors affecting zinc availability. Phosphorus (P) level, nitrogen (N) status, and soil pH seem to be the most important.

Phosphorus

Brinkerhoff (1969) found that high rates of P reduced plant weight, Zn uptake, and yield only when the

Zn supply in the soil was limited. In a greenhouse experiment, Ellis et al. (1964) found that application of 10 pounds of Zn per acre increased the yield of field beans when the treatments included high levels of either applied or residual P. Judy et al. (1964), Ellis (1965), Lessman (1965), and Melton et al. (1970) reported that heavy application of P may induce Zn deficiency, but when Zn was applied at a rate of 4 pounds per acre at each rate of P fertilizer, yields were increased.

Lessman and Ellis (1971) found that the percentage of fertilizer Zn which remained water soluble in the soil increased with increasing rates of Zn until a Zn/P ratio of 1:29 was reached, and then decreased with increasing quantities of ZnO incorporated in ammonium polyphosphate (APP).

Langin et al. (1962) stated that the more effectively the applied P is utilized by the crop, the more severe is the reduction in Zn utilization. On the other hand Boawn et al. (1954) and Seatz et al. (1959) reported that P fertilization did not affect Zn response. Vinande et al. (1968) found that with a high P level, the yield of red kidney beans was increased slightly when Zn was applied.

Langin et al. (1972) and Stukenholtz et al. (1966) stated that the deleterious effect of P on Zn utilization is considered to be largely physiological in nature, probably a plant root adsorption phenomenum, rather than an external Zn-P precipitation.

The actual cause of P-induced Zn deficiency is still unknown, but fortunately the disorder can be alleviated by application of 3 to 4 pounds of Zn per acre in the inorganic form or approximately one-fifth as much Zn in a chelate form (Judy et al. 1964; Ellis 1965; Langin et al. 1962; Brinkerhoff et al. 1967; Melton et al. 1970; and Lindsay 1972).

Nitrogen

The effect of nitrogen (N) fertilizers on the availability of native and applied Zn is related to changes in soil pH, according to Viets et al. (1957). Zinc uptake by three crops of milo and four clippings of Ladino clover was highest when $ZnSO_4$ was applied with ammonium sulphate. In a greenhouse experiment, Ellis et al. (1964) found that an application of ammonium nitrate banded with Zn sulphate resulted in a significantly higher yield and Zn contents of navy beans than when $ZnSO_4$ was banded alone or with monocalcium phosphate or with potassium chloride. Boawn et al. (1960) found that the influence of N carrier on Zn uptake varied with crop grown. They compared ammonium sulfate, ammonium nitrate, and calcium nitrate as N carriers. When an N carrier effect was observed, it was found to be most closely correlated with changes in soil pH, that is, N sources that reduced soil pH increased Zn availability.

Langin et al. (1962) concluded that N has a beneficial action as a controlling factor of Zn deficiency in the corn plant.

Soil Reaction and Carbonates

Recommendations for applications of micronutrients are often based on soil tests, of which soil pH is an important consideration. According to Lucas and Knezek (1972), Zn deficiency in crops is not common on acid soils. Lessman (1967), stated that as soil pH increases as a result of liming, Zn availability decreases.

Camp (1945) stated that the critical pH above which Zn may become unavailable is from 5.5 to 6.5. In the soils studied by Nelson (1956), plants grew normally at pH 5.7 but were chlorotic at pH 7.3.

Terman and Mortvedt (1965) found that Zn deficiency, except in very sandy soils, is not usually a problem below pH 6.0, but noted that the incidence may increase as the pH increases, especially in calcareous soils. They found that the response of corn to Zn was lowered as the soil pH was decreased by the various fertilizers. Hodgson et al. (1966) stated that Zn deficiencies are generally more widespread on calcareous soils.

According to Ellis (1965), in Michigan the most severe Zn deficiency occurs in a region where the soils are usually calcareous; also Ellis (1964) suggested that in this area pH and free calcium carbonate may be related to the occurrence of Zn deficiencies.

Judy (1967) and Langin et al. (1962) state that liming, high pH, and calcareous soils are among the factors which affect the Zn concentration in the plants. Melton et al. (1970) found that heavy P applications generally induced a greater Zn deficiency on soils testing above pH 7.0 which contained free $CaCO_3$. Seatz <u>et al</u>. (1959) noted response by flax and sorghum to Zn fertilization as the rate of liming was increased from 2 to 6 tons of $CaCO_3$ per million pounds of Hartsells soil.

Wear (1953) found that an application of 2000 pounds of $CaCO_3$ per acre considerably decreased the Zn content of sorghum. The pH of the soil was increased from 5.7 to 6.6 and the calcium content of the plants was increased from 0.78 to 1.09 per cent. He concluded that the reduction of Zn uptake by the plants is a pH effect and not a calcium effect.

Soil Tests for Zinc

According to Lindsay (1972), the prime objective of a Zn soil test is to determine whether a given field will show Zn deficiency for certain crops. Bray (1948) proposed that a good soil test should meet the following requirements:

1. The extracting solution and the procedure used should extract the total amount (or

a proportionate part) of the available form or forms of a nutrient from soils with variable properties.

2. The amount of a nutrient in the extract should be measured with reasonable accuracy and speed.

3. The amount of extracted nutrients should be correlated with the growth and the response of each crop to the nutrient under various conditions.

Cox and Kamprath (1972) concluded that eventually the soil test should predict the amount of fertilizer needed to achieve maximum economic production.

Wear and Sommer (1947) found a good correlation between the occurrence of Zn deficiency symptoms and the quantity of Zn extracted with 0.1 N HCl or 0.04 N acetic acid. However, they noted that substituting 0.1 N HCl for 0.04 N acetic acid reduced the time of extraction and the volume of extracting liquid, making it possible to complete a set of determinations within a single working day and reducing the danger of contamination of the reagent. Using a dithizone extraction procedure, Massey (1957) found no correlation between uptake of Zn and total soil Zn.

Barrows and Drosdoff (1966), using a polarographic method for the determination of extractable Zn in mineral soils, obtained significant correlations between extractable Zn in the soil and total Zn in leaves of tung trees.

Martens <u>et al</u>. (1966) working with 57 Wisconsin soils compared four extractants and found that the relative amount of soil Zn extracted was in the order Aspergillus niger > 0.1 <u>N</u> HCl > dithizone > 0.2 <u>M</u> $MgSO_4$. They concluded that much of the additional Zn extracted by 0.1 <u>N</u> HCl as compared to dithizone is not extractable by plants. In most Hawaiian soil profiles, the highest concentrations of Zn extractable with 0.1 <u>N</u> HCl was found to vary from 0.1 to 17.0 ppm and total Zn from 51 to 288 ppm (Kanehiro and Sherman, 1967).

Martens (1968) found that Zn uptake was more closely related to soil Zn extracted with 2 <u>N</u> $MgCl_2$ ($r = 0.663$) than to soil Zn extracted with 0.1 <u>N</u> HCl ($r = 0.297$) or with 1.0 <u>N</u> HCl ($r = 0.301$).

On soils that varied widely in texture and pH, neither 0.1 N HCl nor dithizone-extractable zinc was found to give a reliable estimate of the plant availability of soil Zn. On the other hand, a direct relationship was shown to exist between plant uptake of Zn and soil Zn extracted with 0.1 N HCl on soils of similar texture and pH by Martens and Chesters (1967), and by Massey (1957).

Wear and Evans (1968) extracted Zn from coarse-textured soils with 0.05 N HCl plus 0.025 N H_2SO_4, with 0.1 N HCl, and with 0.05 M EDTA at pH 7.0. The highest correlation for corn and sorghum was obtained with the first extractant. Correlation coefficients (r) for corn for the 3 above extractants were 0.89, 0.82, and 0.62 respectively, and correlation coefficients for sorghum were 0.70, 0.63, and 0.44 respectively.

Extraction with NH_4NO_3, KCl and disodium ethylenediamine di (0 - hydroxyphenol acetic acid) was found by Ravikovitch et al. (1968) to give the most significant multiple correlation coefficients for six different crops growing on 15 different calcareous soils. Melton (1968) reported that a 0.1 N HCl extraction procedure was found to be adequate soil test for plant-available Zn in Michigan.

Trierweiler and Lindsay (1969) developed the EDTA-ammonium carbonate soil test for Zn. This soil test was evaluated on Colorado soils and was compared favorably with the dithizone and 0.1 N HCl methods.

More recently Lindsay and Norvel (1969) reported the use of DTPA as an extractant for diagnosing the Zn, Fe, Mn, and Cu status of soils.

Brown et al. (1971) compared several analytical methods for determining available soil Zn. Soils from 92 fields in California were analyzed for extractable Zn using the DTPA, ammonium acetate-dithizone, 0.1 N HCl, and Na_2EDTA methods. They found a "predictible value" of 83, 79, 73, and 72%, respectively for these tests. On this basis, DTPA was preferable to the other methods.

As cited by Viets and Lindsay (1973), Laner found that the mean labile Zn content of soil determined by corn plants, DTPA extraction and 0.1 N HCl was 4.6, 4.3, and 7.7 ppm of Zn respectively. The labile Zn values in the corn plants and in the DTPA extraction were highly correlated (r^2 = 0.97). Lindsay (1972) concluded that in acid soils, the 0.1 N HCl and the dithizone methods are about equally effective in predicting Zn deficiency. When soils containing $CaCO_3$ are included, the EDTA, DTPA and dithizone extractions are superior to the 0.1 N HCl extraction. The EDTA and DTPA procedures are much more convenient to use than dithizone. The DTPA extractant appears to be one of the more promising soil tests for Zn.

METHODS AND MATERIALS

Soil samples were obtained from 10 locations in Ecuador where field experiments had been conducted, and the results of soil analyses made in the laboratory at Santa Catalina, Quito, Ecuador were available for all except 3 locations (Table 1). Approximately 8 kg of soil was obtained from the Ap horizon from the following locations in the mountain area of Ecuador:

Parroquia	Canton	Provincia	Farm
Aloag	Mejia	Pichincha	Aychapichu
Cutuglahua	Mejia	Pichincha	Santa Catalina
Machachi	Mejia	Pichincha	Chisinche
Tumbaco	Quito	Pichincha	Clementina
Atuntaqui	Antonio Ante	Imbabura	Atuntagui
La Merced	Ibarra	Imbabura	Granja Experimental
Guamani	Quito	Pichincha	Monjas
Pifo	Quito	Pichincha	Alagarin
Cutuglahua	Mejia	Pichincha	El Retiro
El Chaupi	Mejia	Pichincha	Umbria

The soil samples were air-dried, placed in cotton bags, and shipped to Michigan State University. The sam-

Table 1. Chemical analyses of Ecuadorian soils. These determinations were made in the Santa Catalina Experiment Station Laboratory, Quito.

Soils	pH	Al+H	N	P	K	Ca	Mg	Zn	Cu	Fe	Mn
		me/100g	ppm								
Aychapichu	6.2	0.3	45	8	253	1863	169	2.6	3.0	227	3.0
Santa Catalina	5.7	1.0	74	18	115	1069	83	6.1	6.1	377	3.0
Chisinche	6.4	0.3	42	10	278	1306	114	2.2	2.6	113	3.4
Clementina	7.2	0.3	26	2	238	975	269	3.8	2.3	14	2.6
Atuntaqui	---	---	--	--	---	----	---	---	---	---	---
Granja Exp.	---	---	--	--	---	----	---	---	---	---	---
Monjas	6.0	0.3	53	19	221	1013	111	5.9	5.9	475	3.0
Alagarin	---	---	--	--	---	----	---	---	---	---	---
El Retiro	6.5	0.6	71	7	226	1655	329	16.7	5.0	259	1.5
Umbria	6.2	0.3	44	12	182	1281	163	3.6	3.0	196	3.5

ples were treated by steam sterilization by the Quarantine Division of the United States Department of Agriculture in Miami, Florida.

After the soil samples were received at Michigan State University, they were crushed by rolling with the glass bottle, and then stored in cardboard containers.

A sample of a Wisner loam from Bay County, Michigan taken from an area where a marked Zn response was obtained in field experiments was used as a soil for comparison purposes in these experiments.

For the third experiment, samples from 5 Michigan soils varying in texture and fertility level were used; the soil series and locations are listed below:

	Soil Type	County
1.	Nester loam	Clare County
2.	Nester sandy loam	Gladwin County
3.	Kent silt loam	Mason County
4.	Selkirk loam	Mason County
5.	Montcalm sandy loam	Mecosta County

White silica sand from Wedron, Illinois was washed 6 times with 6 N HCl, 3 times with 0.1 N HCl, 3 times with distilled water and 3 times with deionized water, then oven dried and stored in plastic bags. Preliminary cropping (following the procedures given below) to compare washed sand and unwashed sand indicated that the sand did not contain enough plant-available Zn to justify the washing procedure. Consequently all subsequent experiments were conducted using unwashed sand.

Cropping Procedures

At Michigan State University, the Ecuadorian and Michigan soils were cropped in the greenhouse to evaluate their Zn-supplying ability. Six separate experiments were carried out using a cropping procedure adapted from that described by Stanford and DeMent (1957).

Five hundred grams of silica sand were added to 16-ounce wax cartons, into each of which a bottomless 16-ounce carton had been inserted. Fifty ml of Hoagland's nutrient solution without Zn (Hoagland and Arnon, 1950), was added to each container, 6 corn seeds (Var: Mich 500 2X R 121) were spread on the surface and covered with silica sand, which was added until the total weight of the carton was 750 g; last 80 ml of deionized water was added.

After 10 days, a mat of corn roots had developed at the bottom of the inside carton containing the corn seedlings. These cartons with the root mats were removed from the outer cartons and the roots placed on top of 100 g of soil, which was in other 16-ounce waxed cartons. The plants were allowed to grow into the soil for 14 days. For treatments to which Zn was applied, either 5 or 10 ml of 20 ppm solution of Zn as $ZnSO_4 \cdot 7\ H_2O$ was added to the soil prior to cropping. This was equivalent to 10 or 20 ppm Zn, respectively, in the soil. When P was to be added to the soil, 0.209 or 0.418 g of $Ca(H_2PO_4)_2 \cdot H_2O$ was mixed with the soil (to give 500 or 1000 ppm P), and the soil was then wetted to field capacity with deionized water, allowed to air dry, remixed, and then rewetted. In the last experiment the soils were incubated for 12 days after phosphorus was applied; during this time they were wetted to field capacity with deionized water and air-dried at room temperature once every 4 days without further mixing.

Additional nutrient solution was added during the 24-day growing period. After 5, 11, and 18 days, an additional 50 ml of nutrient solution as cited above which did not contain Zn was added. The cultures received deionized water every day to replenish moisture losses.

Each culture also received 3 additional increments of nitrogen (50 ml of 0.005 M $Ca(NO_3)_2$) and iron as Fe citrate (50 ml of a solution containing 6.7 g Fe citrate per liter) when the appearance of the plants indicated a need for N and Fe.

After the mat of roots had been in contact with the soil for 14 days, the corn plants were harvested. The plant material was oven dried at 65 C, weighed, and ground to pass a 20-mesh sieve.

Laboratory Analyses

Total Soil Zinc

Total Zn was determined on the Ecuadorian soil and the Wisner soil by boiling 5 g of soil in 50 ml of 12 N HCl. The suspension was boiled until approximately 5 ml of solution remained and then filtered into 200 ml volumetric flasks. The soil and the filter paper were washed with 1.0 N HCl and deionized water. Zinc was determined using a Perkin Elmer Model 290 Atomic Absorption

Spectrophotometer. This procedure was shown by Melton (1968) to give a reasonable approximation of total soil Zn.

Available Soil Zinc

Available Zn was determined by using the following extraction procedures:

0.1 N HCl.--Soils were extracted using a 1:10 soil:solution ratio, shaking for 10 minutes and filtering.

EDTA-Ammonium Carbonate.--The extracting solution (Trierweiler and Lindsay, 1969) was prepared as follows:

1. Dissolve 32.8 g of EDTA in deionized water and dilute to 1000 ml.

2. Dissolve 1141.1 g of $(NH_4)_2CO_3 \cdot H_2O$ in 500 ml of deionized water.

3. Combine 1 and 2 dilute to 10 liters.

4. The solution was adjusted to a pH 8.6 using HCl or NH_4OH.

Soils were extracted using a 1:2 soil:solution ratio, shaking for 30 minutes and filtering.

DTPA.--The extracting solution (Viets and Lindsay, 1973) was prepared as follows:

1. Dissolve 19.65 g of DTPA (Diethylenetriaminopentacetic acid) in 8 liters of deionized water.

2. Add 5.55 of $CaCl_2$. Mix until dissolved.

3. Add 133 ml of concentrated TEA (Triethanol Amine).

4. Dilute to 10 liters.

5. Adjust the pH to 7.30 using HCl or NH_4OH.

Soils were extracted using a 1:2 soil:solution, shaking for 2 hours and filtering.

Zinc was determined on all solutions using an atomic absorption spectrophotometer.

Routine Soil Tests

At Michigan State University in the soil test laboratory, the Ecuadorian soil samples were routinely analyzed for pH, lime requirement, extractable P, K, Ca and Mh, Zn, Mn and Cu (Table 2). Using the procedure described below:

<u>Soil pH.</u>--Ten grams of soil were mixed with 10 ml of water (1:1 ratio). After 15 minutes, the mixture was stirred again, and the pH of the suspension determined using a glass electrode pH meter. The lime requirement of samples testing below pH 6.8 was determined by the method of Shoemaker, McClean, and Pratt (1961).

<u>Extractable Phosphorus</u>.--Phosphorus was extracted for 5 minutes from samples with Bray P-1 reagent (0.025 $\underline{N}$ HCl and 0.03 $\underline{N}$ NH_4F), using a 1:8 soil:solution ratio. Phosphorus in the extract was determined by using the Spectronic 20 colorimeter at 880 m and the ascorbic acid reduced blue color described by Watanabe and Olsen (1965).

<u>Extractable Potassium, Calcium, and Magnesium.</u>--Cations were extracted for 5 minutes with 1.0 $\underline{N}$ NH_4OAc (pH 7.0) using a 1:8 soil:solution ratio (Jackson, 1958). Potassium, Ca and Mg in the extract were determined by means of a Perkin Elmer Model 290 atomic absorption spectrophotometer.

Table 2. Chemical analysis of Ecuadorian soils. These determinations were made in the Michigan State University Laboratory.

Soils	pH	P	K	Ca	Mg	Zn	Mn	Cu
		ppm						
Aychapichu	5.7	12	156	1014	129	8.8	65	12
Santa catalina	5.5	16	180	1014	94	10.4	72	24
Chisinche	5.8	19	126	883	94	6.8	39	10
Clementina	6.8	7	138	818	310	6.0	56	9
Atuntaqui	7.5	6	318	1344	379	5.6	73	13
Granja Exp.	7.8	39	132	2785	645	10.0	125	10
Monjas	5.6	45	342	752	112	9.6	65	15
Alagarin	5.8	9	222	883	237	9.2	64	10
El Retiro	6.3	13	114	1278	323	11.2	53	10
Umbria	5.7	28	168	1014	177	12.0	55	11

Available Zinc and Manganese.--Available Zn and Mn were extracted for 10 minutes with 0.1 N HCl using a 1:10 soil:solution ratio. (Nelson, *et al*., 1959). Zinc and Mn in the extract were determined by means of a Perkin Elmer Model 290 atomic absorption spectrophotometer.

Available Copper.--Available Cu was extracted for 1 hour with 1.0 N HCl using 1:10 soil:solution ratio. Copper in the extract was determined by means of a Perkin Elmer Model 290 atomic absorption spectrophotometer.

Plant Analyses

One g of plant material was ashed in a muffle furnace at 550 C for 8 hours. The ashed samples were treated with 10 ml of 1.0 N HCl, and the resulting solution filtered into 100 ml volumetric flasks and washed with deionized water. Zinc in solution was determined using the atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

Six separate experiments were conducted in the greenhouse; the first 3 to develop the cropping technique, and the last 3 to evaluate the relative Zn status of 10 Ecuadorian soils. The pots were systematically arranged in four replications. The data for yields, Zn content of the tissue, and Zn uptake per pot were analyzed by means of the analysis of variance, even though it was recognized that this was not completely justified since the pots were not arranged randomly.

Development of the Cropping Technique

First Experiment

Three levels of Zn (none, 10, and 20 ppm) were applied to a Wisner loam which was known to be deficient in Zn from the results of previous field experiments.

<u>Yields.</u>--Yields were significantly higher when 20 ppm Zn was applied than when no Zn was applied, but were not significantly higher when 10 ppm was applied (Table 3).

Table 3. Yield, concentration, and uptake of zinc by corn plants grown on a Wisner loam without applied zinc and with 10 and 20 ppm.

Rate of Applied Zn	Yield	Zinc in Plants	
		Concentration	Total uptake
ppm	g/pot	ppm	mg/pot
0	2.50	16	0.045
10	2.60	31	0.082
20	2.66	23	0.064
LSD (0.05)	0.12	NS	0.028
LSD (0.01)	NS	NS	NS
CV (%)	.23	2.51	2.10

<u>Zinc Content of Tissue.</u>--Concentration of Zn (ppm) tended to increase in the tissue when Zn was applied, but this increase was not significant. The highest concentration of Zn was obtained when 10 ppm of Zn was added to the soil (Table 3).

Uptake of Zinc.--The uptake of Zn (mg Zn/pot) was calculated as follows:

$$\text{mg Zn/pot} = \frac{\text{Weight of plants x ppm of zinc in plants}}{1000}$$

The uptake of Zn was significantly increased when Zn was applied to the soil as compared to the treatment in which no Zn was applied (Table 3). Uptake was less, but not significantly less, when 20 ppm Zn was applied than when 10 ppm was applied; this decrease is a reflection of the lower Zn content of the tissue when 20 ppm was applied.

Second Experiment

Wisner loam was again used from the same location as that used in the first experiment, and 3 levels of Zn (none, 10 and 20 ppm) were applied at each of two levels of phosphorus (none and 500 ppm P).

Yields.--When no P was applied, no significant yield increases were obtained when Zn was applied, although the yield tended to be higher when 10 ppm Zn was applied (Table 4). When P was applied, the yield was significantly higher when 20 ppm Zn was applied than when no Zn was applied, and the yield when 10 ppm was applied tended to be higher. The highest yield was obtained with the highest levels of both Zn and P, and the lowest yield when P was applied without Zn.

Table 4. Yield, concentration, and uptake of zinc by corn plants grown on a Wisner loam with 0, 10, and 20 ppm applied zinc at levels of 0 and 500 ppm applied phosphorus.

Rate of Applied P	Rate of Applied Zn	Yield	Zinc in Plants	
			Concentration	Total Uptake
ppm	ppm	g/pot	ppm	mg/pot
0	0	3.15	42	0.133
0	10	3.23	54	0.174
0	20	3.08	65	0.199
500	0	2.95	39	0.120
500	10	3.06	48	0.153
500	20	3.46	61	0.210
LSD (0.05)		0.30	4	0.013
LSD (0.01)		NS	6	0.018
CV (%)		0.26	0.20	0.22

<u>Zinc Content of Tissue.</u>--The concentration of Zn in the tissue increased with each increasing increment of Zn at both levels of P (Table 4). At each level of applied Zn, the concentration of Zn in the tissue was significantly higher when no P was applied, than when P was applied; Zn tended to be higher when neither Zn nor P were applied than when only P was applied.

<u>Uptake of Zinc.</u>--Zinc uptake increased with each increment of applied Zn at both P levels (Table 4). The uptake of Zn with none or 10 ppm Zn was higher when no P was applied than when P was applied. No difference in Zn uptake due to P level was noted when 20 ppm Zn was applied, due apparently to the yield levels obtained for these two treatments.

<u>Third Experiment</u>

Five different Michigan soils together with the Wisner reference soil were cropped as before without Zn applications, except that the same 3 levels of Zn (0, 10 and 20 ppm) were applied to Wisner loam. Two check treatments in which sand was used instead of soil were also included.

Yields.--Yields increased as the rate of Zn increased on Wisner loam. The lowest yield was obtained with Nester loam and the highest with Nester sandy loam (Table 5). No other significant differences were noted between soils when no Zn was applied or when sand was used instead of soil.

Zinc Content of Tissue.--Zinc concentration was significantly higher when Zn was applied on Wisner loam than when no Zn was applied (Table 5). Using the Wisner soil to which no Zn was applied as the reference for comparison, significantly higher Zn concentrations were noted with Nester loam and with the Kent and Selkirk soils.

Uptake of Zinc.--Uptake of Zn was significantly increased as each increment of Zn was applied to Wisner loam (Table 5). The higher uptake of Zn when sand was used instead of soil cannot be explained, but it does illustrate the need for extreme care in both the cropping and analytical procedures to prevent Zn contamination. Again using the Zn deficient Wisner soil as a standard for comparison, no differences in Zn uptake were noted between the Wisner soil and Nester sandy loam. The Kent

Table 5. Yields, concentration, and uptake of zinc by corn plants grown on 5 different Michigan soils, Wisner loam without applied zinc and with 10 and 20 ppm applied zinc, and sand.

Treatments			Zinc in Plants	
Soil	Rate of Applied Zn	Yield	Concentration	Total Uptake
	ppm	g/pot	ppm	mg/pot
Nester loam	0	1.57	55	0.086
Nester sandy loam	0	2.00	38	0.076
Kent silt loam	0	1.80	45	0.081
Selkirk loam	0	1.83	49	0.089
Montcalm sandy loam	0	1.89	40	0.075
Wisner loam	0	1.83	42	0.076
Wisner loam	10	1.95	63	0.121
Wisner loam	20	2.00	65	0.129
Sand	0	1.83	48	0.087
Sand	0	1.89	51	0.096
LSD (0.05)		0.33	3	0.006
LSD (0.01)		NS	4	0.008
CV (%)		0.31	0.14	0.11

soil, or the Montcalm soil, suggesting that these soils might also be deficient in Zn.

Discussion of Cropping Technique

The results of these first 3 experiments indicate that differences in the uptake of Zn can be obtained using the modified Stanford-Dement cropping technique. The results of the first 2 experiments (Table 3 and 4) show that differences in uptake can be obtained when different rates of Zn are applied to the same soil. The third experiment (Table 5) demonstrates that differences can be obtained in Zn uptake between different soils to which no Zn is applied. The total dry weight of the plants, the concentrations of Zn in the tissue and the total uptake of Zn varied considerably between the 3 experiments. This illustrates the necessity of following a carefully controlled cropping technique in conducting experiments of this type. Furthermore, a soil for comparison, known to be deficient in Zn, must be included in each experiment to establish a level of Zn uptake for comparison. Usually, a level of 20 ppm Zn in plant tissue is considered to be the critical level below which growth responses to added Zn can be expected. Concentration of Zn in the plants grown in these experiments was above this level in the second and third experiments. This further illustrates the necessity of including a soil of known Zn response for comparisons.

Evaluation of Zinc Status of Ecuadorian Soils By Cropping in the Greenhouse

Fourth Experiment

In this experiment, the Zn status of 10 Ecuadorian soils was evaluated. The Zn-deficient Wisner soil and another treatment in which sand was used instead of soil were included for comparison.

Yields.--The highest yield was obtained with the Monjas soil, which was the only soil with which the yield was significantly different from that with the Wisner soil. The lowest yield was obtained with the sand culture without soil (Table 6).

Zinc Content of Tissue.--The highest concentration of Zn was in plants grown on Aychapichu soil and the lowest in those on the Granja Experimental soil (Table 6). The concentration of Zn was higher in plants grown on all soils than on Wisner soil except for the Clementina, Granja Experimental, and Monjas soils, from which the Zn concentration in plants was not different from that in plants grown in Wisner soil.

Uptake of Zinc.--The highest uptake of Zn was obtained with the Aychapichu soil and the lowest in the Wisner soil (Table 6). On 5 soils--Clementina, Atuntaqui, Granja Experimental, El Retiro, and Umbria--the uptake of Zn was not significantly different from that with Wisner soil. This suggests that these soils may also be deficient in Zn.

Fifth Experiment

The preceding experiment was repeated in order to check the reproducibility of the results.

Yields. Yields obtained on 3 soils--Chisinche, Clementina, and Monjas--were significantly higher than that obtained on Wisner soil (Table 7). Yields on all soils were significantly higher than that with sand.

Table 6. Yields, concentration, and uptake of zinc by corn plants grown on Ecuadorian soils, Wisner loam soil and sand.

Soil	Yield	Zinc in Plants	
		Concentration	Total Uptake
	g/pot	ppm	mg/pot
Aychapichu	2.83	52	0.147
Santa Catalina	2.73	48	0.131
Chisinche	2.86	43	0.123
Clementina	2.82	40	0.113
Atuntaqui	2.70	38	0.103
Granja Exp. Imbabura	2.67	34	0.099
Monjas	2.92	41	0.120
Alagarin	2.50	45	0.121
El Retiro	2.55	46	0.117
Umbria	2.52	43	0.109
Wisner soil	2.58	38	0.098
Sand	2.31	45	0.093
LSD (0.05)	0.30	5	0.019
LSD (0.01)	NS	7	0.025
CV (%)	0.16	0.16	0.24

Table 7. Yield, concentration, and uptake of zinc of corn plants grown on Ecuadorian soils, Wisner loam soil and sand.

Soil	Yield	Zinc in Plants	
		Concentration	Total Uptake
	g/pot	ppm	mg/pot
Aychapichu	2.05	46	0.094
Santa Catalina	2.10	51	0.106
Chisinche	2.15	42	0.090
Clementina	2.16	41	0.088
Atuntaqui	1.82	38	0.069
Granja Exp. Imbabura	2.10	39	0.082
Monjas	2.21	50	0.110
Alagarin	1.94	42	0.081
El Retiro	1.99	52	0.103
Umbria	1.97	58	0.114
Wisner soil	1.94	40	0.077
Sand	1.54	38	0.058
LSD (0.05)	0.19	3	0.011
LSD (0.01)	0.26	4	0.014
CV (%)	0.13	0.10	0.17

Zinc Content of Tissue.--The highest concentration of Zn was found in the Umbria soil, and the Zn concentration in this soil, together with Aychapichu, Santa Catalina, Monjas, El Retiro, was significantly higher than that from the Wisner soil (Table 7).

Uptake of Zinc.--The greatest uptake of Zn was obtained with the Umbria soil, on which the uptake was not significantly different from Wisner in the fourth experiment. The uptake of Zn with the Atuntaqui, Granja Experimental and Alagarin soils was not significantly different from that with Wisner (Table 7). Except for the Alagarin and Umbria soils, these results are in agreement with those obtained in the preceding experiment.

Sixth Experiment

This experiment was conducted concurrently with the preceding experiment, but 1000 ppm of phosphorus was applied to each of the Ecuadorian soils and to the Wisner soil. A treatment in which sand was used instead of soil was also included.

Yields.--Yields obtained with the Chisinche and El Retiro soils were significantly greater than that with the Wisner soil (Table 8). Yields on all soils except the Clementina soil were significantly more than that with sand.

Zinc Content of Tissue.--The highest concentration of Zn was in plants grown on the Santa Catalina soil. The concentration of Zn was higher on the Aychapichu, Santa Catalina, Monjas, El Retiro and Umbria soil than in the Wisner soil (Table 8). The Atuntaqui soil had the lowest concentration of zinc.

Uptake of Zinc.--The highest uptake of Zn was obtained with the Santa Catalina soil and the least with the Atuntaqui (Table 8). The uptake of Zn from the Clementina, Atuntaqui, Granja Experimental and Alagarin soils was either not different or lower than that from the Wisner soil. Uptake of Zn from all soils except Clementina and Atuntaqui was significantly higher than that from Wisner soil.

Relation of Yield and Zinc Concentration to Zinc Uptake

Yield and Zn content were not correlated in any of the last 3 experiments (Table 9), nor were yield and

Table 8. Yield, concentration, and uptake of zinc by corn plants grown on Ecuadorian soils, Wisner loam soil and sand with 1000 ppm of phosphorus applied.

Soil	Yield	Zinc in plants	
		Concentration	Total Uptake
	g/pot	ppm	mg/pot
Aychapichu	2.39	38	0.090
Santa Catalina	2.54	39	0.099
Chisinche	2.69	34	0.091
Clementina	2.17	29	0.062
Atuntaqui	2.42	25	0.060
Granja Exp. Imbabura	2.49	30	0.074
Monjas	2.55	36	0.091
Alagarin	2.49	28	0.069
El Retiro	2.65	36	0.094
Umbria	2.56	36	0.092
Wisner soil	2.33	32	0.074
Sand	1.85	29	0.053
LSD (0.05)	0.26	4	0.013
LSD (0.01)	0.36	6	0.017
CV (%)	0.16	0.18	0.23

Zn uptake except in the sixth experiment. Zinc concentration and uptake were highly correlated (0.01 level) in all 3 experiments (Table 9).

Table 9. Linear correlation coefficients (r) between yield, concentration of zinc, and uptake of zinc on the Ecuadorian and Wisner soils.

Comparisons	Experiment 4	Experiment 5	Experiment 6
Yield vs Zn content	0.050	0.148	0.424
Yield vs uptake	0.380	0.477	0.689*
Zn content vs uptake	0.910**	0.938**	0.936**

*Significant at 0.05 level
**Significant at 0.01 level

This means that actual yields were not as greatly affected by Zn level in soil as was the concentration of Zn in the plant. Also, differences in Zn status of soils are reflected by differences in Zn absorption by the plants, not in growth. This would be expected since the concentrations of Zn in the plants was always considerably higher than the generally accepted critical level of 20 ppm. Either concentration or total uptake could be used to evaluate Zn status of soils. However, Zn uptake will be used herein as a means of evaluating the Zn status of the soils, since it was felt that this value would probably reflect the Zn level in the soils more accurately than concentration should some other factor affect yield.

Relation Between Available Zinc and Soil pH

Available Zn extracted with 0.1 N HCl was not correlated with soil pH (Table 10). Significant (0.05

Table 10. Linear correlation coefficients (r) between 0.1 N HCl, EDTA, and DTPA Zn extraction procedures and pH on the Ecuadorian soils.

Comparisons	Correlation coefficient r
pH vs 0.1 N HCl	-0.29
pH vs EDTA	-0.69*
pH vs DTPA	-0.62*

*Significant at 0.05 level.

level) correlations were noted between soil pH and Zn extracted with EDTA and DTPA, in which level of Zn decreased as pH increased. However, these correlations were not as high as those obtained between Zn uptake by cropping and the Zn extracted by these two methods.

Zinc Status of Ecuadorian Soils

In all 3 of the experiments with Ecuadorian soils (Tables 6, 7, and 8), the uptake of Zn from the Atuntaqui, Clementina, and Granja Experimental soils was not significantly different from Zn uptake from the Zn-deficient Wisner soil. This would suggest that those soils might be deficient in Zn, and so would be the soils on which further field experiments would be warranted.

Evaluation of Procedures for Measuring Available Soil Zinc

Total Zinc

Total zinc was extracted from the soil with 12.0 N HCl. More Zn was extracted from the Wisner soil than from any of the Ecuadorian soils (Table 11). The amounts

Table 11. Amount of zinc extracted from Ecuadorian and Wisner soils by four extractants (ppm).

Soils	12.0 N HCl	0.1 N HCl	EDTA	DTPA
Aychapichu	53.3	7.73	4.37	3.07
Santa Catalina	57.6	9.33	6.69	3.95
Chisinche	46.5	4.93	2.74	1.47
Clementina	52.6	3.60	0.80	0.51
Atuntaqui	37.3	3.73	0.74	0.48
Granja Exp.	57.1	8.13	2.10	1.36
Monjas	42.9	7.46	4.56	2.75
Alagarin	48.5	4.80	2.29	1.44
El Retiro	52.8	9.73	5.44	4.08
Umbria	43.4	7.60	4.64	2.96
Wisner loam	69.3	5.60	1.76	1.17

of total Zn extracted from the Ecuadorian soils varied from 57.6 ppm in the Santa Catalina soil to 37.3 ppm in the Atuntaqui soil. Total Zn was not correlated with either Zn uptake (Table 12) or with the "available" Zn extractions (Table 13).

Table 12. Linear correlation coefficients (r) between soil tests and uptake of Zn in the 3 experiments with Ecuadorian and Wisner soils.

Comparisons	Experiment 4	Experiment 5	Experiment 6
12.0 N HCl vs uptake	0.090	0.150	0.060
0.1 N HCl vs uptake	0.208	0.676*	0.790**
EDTA vs uptake	0.476	0.704*	0.925**
DTPA vs uptake	0.469	0.813**	0.893**

*Significant at 0.05 level
**Significant at 0.01 level

Table 13. Linear correlation coefficients (r) between total zinc and available Zn in Ecuadorian soils as measured by 3 extraction procedures.

Comparisons	r
12.0 N HCl vs 0.1 N HCl	0.26
12.0 N HCl vs EDTA	0.05
12.0 N HCl vs DTPA	0.07
0.1 N HCl vs EDTA	0.87**
0.1 N HCl vs DTPA	0.90**
EDTA vs DTPA	0.98**

**Significant at 0.01 level.

Available Soil Zinc

0.1 HCl Extraction.--The amount of Zn extracted from the Ecuadorian soils varied from 9.73 ppm in the El Retiro soil to 3.73 ppm in the Atuntaqui soil (Table 11). Of the 3 soils which were considered to be potentially Zn deficient from the cropping experiments, lower levels of extractable Zn were obtained from the Atuntaqui and Clementina soils than for the Wisner soil, but a higher level was noted for the Granja Experimental soil. Also the

Chisinche and Alagarin soils have lower levels of Zn than the Wisner soil.

Linear correlation analyses between Zn extracted with 0.1 N HCl and Zn uptake during cropping (Table 12) showed no correlations in the fourth experiment, a significant correlation in the fifth experiment, and a highly significant correlation in the sixth experiment when P was applied.

EDTA Extraction.--The amount of Zn extracted varied from 6.69 ppm in the Santa Catalina soil to 0.74 ppm in the Atuntaqui soil. Of the 3 potentially Zn-deficient soils, lower levels of extractable Zn were noted from the Atuntaqui and Clementina soils, but a slightly higher level was obtained for the Granja Experimental soil. All of the other soils also had higher levels of extractable Zn than the Wisner soils. Less Zn was extracted from the soils with EDTA than with 0.1 N HCl.

The linear correlation coefficient between extractable Zn and uptake of Zn was not significant for the fourth experiment (Table 12), but significant coefficients were obtained in the fifth and sixth experiments.

Again, a higher correlation was obtained when P was applied to the soils than when no P was applied.

DTPA Extraction.--The amount of Zn extracted with DTPA varied from 4.08 ppm in the El Retiro soil to 0.48 ppm in the Atuntaqui soil. Of the 3 potentially Zn-deficient soils, less Zn was extracted from the Atuntaqui and Clementina soils than from the Wisner soil, and again slightly more was extracted from the Granja Experimental soil. Also, higher Zn levels were obtained for all the other soils than from the Wisner soil. Less Zn was extracted from the soils with DTPA than with either 0.1 *N* HCl or EDTA.

The linear correlation between Zn uptake and Zn extracted with DTPA was not significant for the fourth experiment, but highly significant correlations were obtained for both the fifth and sixth experiments (Table 12). Again, a higher correlation was obtained when P was applied.

Evaluation of Available Zinc Extractants

The total amount of Zn in the soils does not appear to be related to the level of available Zn (Tables 12 and 13). All 3 of the methods for determining available soil Zn were correlated with Zn uptake by cropping (Table 12), indicating that any one of the 3 could probably be successfully correlated with yield response to Zn. The correlation coefficient of 0.98 between EDTA and DTPA extractable Zn (Table 13) indicates an almost perfect correlation between these 2 extractants; this would be expected since both are complexing or "chelating" agents which would be expected to react similarly with soil Zn. On the basis of these results, either of these two extractants would probably be somewhat more satisfactory for routine testing than 0.1 N HCl. Since the DTPA extracting solution is easier to make up in the laboratory and is less susceptible to decomposition with time, it is probably the most practical for routine use.

CONCLUSIONS

The short-term cropping procedure developed by Stanford and Dement (1957) can be used to measure differences in Zn uptake by corn from soils having different levels of available soil Zn, or from soils to which different levels of Zn have been applied. However, the techniques used in following this procedure must be standardized and carefully followed in each experiment if reproducible results are to be obtained. Since the plants are grown for only a short time (2 weeks in contact with soil), growth differences are not obtained so that comparisons must be made between the total Zn uptake from the different soils. In order to separate soils which may respond to Zn applications in the field from those which will not respond, a soil known to be deficient must be included in all experiments as a means of comparison.

Using this technique, Zn uptake from 10 Ecuadorian soils was determined and uptake from 3 of the 10

(Atuntaqui, Clementina, and Granja Experimental) was either lower or not different from Zn uptake from the Zn deficient soil (Wisner loam). This suggests that these soils may be deficient in Zn, and that field experiments are justified on these soils.

The uptake of Zn from these soils was correlated with Zn extracted using 3 different extracting solutions: 0.1 N HCl, EDTA, and DPTA. Any one of the 3 extractants could be satisfactorily used as a soil test for available Zn. It is suggested that DTPA would be the most satisfactory extractant to use, since slightly higher correlations were obtained with DTPA than the other extractants, and since the solution seems to be more adapted to laboratory routine procedures than the EDTA solution.

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