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DIVISION S-4—SOIL FERTILITY AND PLANT NUTRITION

Correlation Between Available Soil Arsenic, Estimated by Six Methods, and Response of Corn (*Zea mays* L.)[1]

E. A. WOOLSON,[2] J. H. AXLEY,[3] AND P. C. KEARNEY[2]

ABSTRACT

The results of six methods of estimating available As on 28 different soils from eight states in the United States were correlated with the wet tissue weight of 4-week-old corn (*Zea mays* L.) grown on these soils. Linear correlation coefficients between plant weight and the log of the soil As concentration were determined by the following techniques (*i*) total As; (*ii*) summation of "Easily-Soluble-," Fe-, Al-, and Ca-arsenate fractions; (*iii*) summation of "Easily-Soluble-," Fe-, Al-, and Ca-arsenate fractions modified by their individual phytotoxicity; (*iv*) 0.05*N* HCl and 0.025*N* H_2SO_4 mixed-acid-extractable As; (*v*) 0.5*N* $NaHCO_3$-extractable As; and (*vi*) selection of the As extracted by 0.05*N* HCl and 0.025*N* H_2SO_4 or 0.5*N* $NaHCO_3$ methods, depending on soil pH. The last four techniques were equally effective in predicting plant response ($r = 0.81$ to 0.82) to soil arsenic. The mixed-acid or bicarbonate methods were preferred because they require less labor.

Additional Key Words for Indexing: soil arsenic, soil test, As phytotoxicity.

ARSENIC is used as a herbicide, insecticide, soil sterilant, silvicide, and desiccant in this country. Arsenic has also been introduced into the environment in fertilizers and flue gases from copper smelting. As a result, As may accumulate in soil in some cases (2, 4, 17, 18). When the As level increases, the chances of a phytotoxic response by the desired crop also increases. Therefore, a method for estimating the amount of available As present in the soil and correlating this value to plant growth is desirable.

Estimating soil fertility or plant nutrient availability from the results of chemical methods has been extensively used. The phytotoxicity or availability of As may also be determined by such a soil test.

Available As in soils has been determined with varying degrees of success. Albert and Arndt (1) reported that soluble As, when measured by a colloidion bag dialysis, was a more reliable index of phytotoxicity than total As. Vandecaveye et al. (18) found no correlation between phytotoxicity and the solubility of soil As in distilled water, 0.1*N* HN_4Ac, 0.1*N* NH_4NO_3, 0.1*N* KNO_3, or hot concentrated HNO_3. However, Vandecaveye (17) later reported that As not soluble in 0.1*N* NH_4Ac had little injurious effect on plant growth. Reed and Sturgis (10) reported that a better correlation existed between plant growth and 0.5*N* HCl extractable As than with water-soluble As. Because of the lack of a good method for determining available As and because of chemical similarities between As and P, a review of methods for determining available P may be helpful.

Williams and Cooke (20) compared seven extractants by correlating P extracted with plant response. In these experiments, 0.5*N* $NaHCO_3$-extracted P (8) correlated best with plant response. In the same study, 0.3*N* HCl (13), 0.002*N* H_2SO_4 (15), and acetic acid-sodium acetate (HAc-NaAc, pH = 4.8) buffer solution (14) methods were equally effective, though inferior to $NaHCO_3$. Three extractants [0.5*N* HAc (21), 1% citric acid (H_3Ci), 0.1*M* $CaCl_2$ (11)] were of little general use. The $NaHCO_3$ soluble P did not correlate well on all crops, especially when no P was added to the soil. Warren and Johnston (19) and Boyd (3) likewise found that P extracted with 0.5*N* $NaHCO_3$ gave best correlation with plant response on a majority of soils studied.

Miller and Axley (6), working on 17 acid Maryland soils studied six methods of extracting available phosphorus. They found very little difference between the correlation coefficients for the various chemical methods (including 0.5*N* $NaHCO_3$) when the extracted phosphorus was compared to the yield of alfalfa (Medicago sativa).

Available P is related to the form of P found in the soil. Soil test P is highly correlated with Al-P by Shelton and Coleman (13). Juo and Ellis (5) also examined chemical and physical forms of Al-P and Fe-P in reference to plant uptake. They found that P from colloidal Al-P was more available to sudangrass (*Sorghum sudanense*) than P from colloidal Fe-P. Crystallized forms also followed this order of availability between Fe-P and Al-P but were less available than their colloidal forms.

The present study determined the phytotoxicities of Fe-, Al-, Na-, and $Ca(H_2AsO_4)_x$ compounds in soil and compared six techniques of estimating As phytotoxicity (or availability) in soils.

MATERIALS AND METHODS

Phytotoxicity Factors

Two compounds, $Fe(H_2AsO_4)_3$ and $Al(H_2AsO_4)_3$ were prepared by gradually adding 100 ml of 1*M* NaH_2AsO_4 to 100 ml of 1*M* $FeCl_3$ or $AlCl_3$ with constant stirring and heating to 95C. The pH was maintained at 4.5 ± 0.5 with *N* NaOH or *N* HCl. The samples were heated for 24 hr. The precipitates were filtered and washed with hot distilled water until free from Cl^-. Two other compounds, NaH_2AsO_4 and $Ca(H_2AsO_4)_2$, were purchased commercially.

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[2] Analytical Chemist and Research Chemist, respectively: Crops Research Div., ARS, USDA, Beltsville, Md. 20705.

[3] Professor, Dept. of Agronomy, Univ. of Maryland, College Park, Md.

9137

202 JILL PODOLSKY, EHA PAGE 06

OTR POP HEALTH PAGE 02

Each As compound (Na^+, Fe^{3+}, Al^{3+}, or Ca^{2+} $(H_2AsO_4)_x$) was incorporated into 300 g of Lakeland loamy sand at a rate equivalent to 0.01, 0.1, 1.0, 10, or 100 μg As/gram of soil with three replications. Three corn plants (*Zea mays*, 'US 13') were grown on each soil for four weeks, harvested, and the fresh weight determined.

Methods for Extracting and Determining Arsenic

Arsenic was determined in soil extracts and digests by the colorimetric method reported by Woolson (22). The method consisted of an arseno-molybdum blue color developed on a reduced and distilled As solution. The As^{3+} was trapped in a KI-I_2 solution and the color reagents, hydrazine sulfate and ammonium molybdate, added. The color was developed in a hot water bath and read at 840 mμ. A thousand-fold excess of phosphate and sulfate in the starting sample solution did not interfere with the As determination.

Total As—Dry soil samples (1.00 g) were digested with 6 ml of $HClO_4$ and H_2SO_4 acids (1:1, v/v) (22).

Summation of Chemical Fractions—Easily soluble-, Fe-, Al-, and Ca- forms of soil As were extracted according to the modified Chang and Jackson procedure as described by Peterson and Corey (9), and the amounts found were summed.

Modified-Summation of Chemical Fractions—The amount of easily soluble-, Fe-, Al-, and Ca-arsenates found by the preceding method was multiplied by their respective phytotoxic factor. These modified fractions were summed (Σ = 1.0 × E. S. + .62 × Al-As + .54 × Ca-As + .13 × Fe-As).

Mixed-Acid-Extractable As—Arsenic was extracted with 0.05*N* HCl and 0.025*N* H_2SO_4 as described by Nelson et al. (7).

Bicarbonate-Extractable As—Arsenic was extracted with 0.5*N* $NaHCO_3$ as described by Olsen et al. (8).

Selection of As extracted by the Acid or Bicarbonate Methods Depending on pH—Arsenic was extracted by the mixed acid (0.05*N* HCl and 0.025*N* H_2SO_4) when soil pH < 6.5 or by 0.5*N* $NaHCO_3$ when soil pH ≥ 6.5. Soil pH was determined by using 1:1 *N* KCl.

Bioassay for Phytotoxic Arsenic

Twenty-nine arsenic contaminated soils (Table 1) from eight states were sampled at a depth of 0–15 cm. A second apparently untreated sample from the same soil type was also taken and used as a check. Three hundred grams of soil was placed in cups and fertilized at a rate of 134 Kg N/ha by wt. Four replications of these paired soils were arranged in a completely randomized block design in the greenhouse. Three corn plants (*Zea mays* L., US 13) were grown for 4 weeks, harvested, and weighed. The plant weights from the contaminated soils were compared with plant weights from the uncontaminated soils to determine the degree of As phytotoxicity.

RESULTS AND DISCUSSION

The phytotoxicity of Na-, Fe-, Al-, and Ca-As compounds in Lakeland loamy sand is shown by the percent

Table 1—Some soil properties of soil tested

Soil number, name and location		pH	Available cations Ca*	Fe†	Al‡
			meq/100 g		
Mississippi Cahaba fsl	7 a	5.44	2.6	342	6.7
	b	5.54	2.9	698	4.0
Norfolk sl	8 a	5.80	2.7	327	-
	b	5.72	2.6	396	4.3
Memphis sil	9 a	5.40	3.2	1,057	6.3
	b	5.62	4.0	934	6.6
Alabama Chesterfield sil	11 a	--	-	-	-
	12 b	5.61	3.4	369	4.0
	13 b	5.40	3.2	417	5.2
	14 b	5.52	3.0	386	4.7
	15 b	5.44	3.6	392	6.4
	16 b	5.37	3.2	472	6.2
Idaho Greenleaf sl	22 a	7.50	8.5	1,078	2.5
	b	7.23	11.9	987	3.9
	23 a	7.11	8.6	925	3.3
	b	7.01	8.2	906	4.0
	24 a	--	-	-	2.4
	b	6.35	9.4	866	3.6
	25 a	--	-	-	3.0
	b	6.16	8.7	856	4.6
Oregon Agate gr)	70 a	--	-	-	2.8
	b	5.78	8.5	1,155	2.7
New York Newfane sl	38 a	--	-	-	5.9
	b	4.40	1.8	745	4.8
Nilson sil	39 a	--	-	-	2.9
	b	5.28	5.2	728	4.5
Dunkirk sil	40 a	--	-	-	4.4
	b	4.62	2.2	712	6.3
Lockport sicl	41 a	--	-	-	9.5
	b	4.40	1.2	886	13.5
Dunkirk fs	42 a	4.89	2.6	785	22.1
	b	4.99	2.2	799	10.2
Florida Lakeland fs	48 a	--	-	-	-
	b	7.14	6.9	35	1.5
	c	7.14	9.5	35	1.3
Indiana Unidentified (a loess soil)	49 a	--	-	-	2.9
	b	5.97	2.6	271	2.0
	50 a	--	-	-	2.9
	b	4.40	1.4	572	3.6
	51 a	--	-	-	2.2
	b	4.82	0.8	277	2.2
	53 a	--	-	-	2.1
	b	5.07	2.6	200	2.0
	55 a	6.85	12.6	1,573	3.8
	b	6.10	8.2	1,786	8.8
	c	4.99	4.4	1,739	4.6

* Sodium acetate extraction followed by ion specific electrode analysis (23).
† Oxalate-oxalic acid extraction followed by orthophenanthroline detection at 540 mμ (12).
‡ Sodium hydroxide extraction followed by pH adjustment after adding NH_4F (24, 25).

Table 2—Total arsenic and percentage of total arsenic in the form of easily soluble, iron, aluminum, and calcium arsenates present in soil samples*

Soil number	Arsenic Total	Easily sol.	Al-As	Fe-As	Ca-As	Growth reduction
	ppm	%	%	%	%	
7 a†	28	0	24	76	0	-
b	81	0	17	83	0	10
8 a	22	0	7	93	0	-
b	53	0	14	77	9	-4
9 a	21	0	0	91	9	-
b	96	0	13	76	12	-13
11 a	8	0	33	67	0	-
12 b	16	0	31	62	7	-28
13 b	80	0	45	48	7	13
14 b	120	0	52	48	0	35
15 b	24	0	31	54	15	-26
16 b	28	0	53	35	13	-15
22 a	16	-	-	-	-	-
b	138	9	32	42	17	32
23 a	28	0	0	0	0	-
b	170	7	34	46	13	69
24 a	10	-	-	-	-	-
b	204	4	35	46	15	66
25 a	6	-	-	-	-	-
b	162	11	29	47	13	72
27 a	27	0	0	100	0	-
b	54	0	24	49	27	50
28 a	5	-	-	-	-	-
b	81	0	14	55	31	15
70 a	10	-	-	-	-	-
b	67	0	22	56	22	-5
38 a	4	-	-	-	-	-
b	319	0	24	63	13	42
39 a	6	-	-	-	-	-
b	132	0	31	55	14	8
40 a	4	-	-	-	-	-
b	252	0	17	69	14	37
41 a	3	-	-	-	-	-
b	90	0	19	75	6	40
42 a	12	-	-	-	-	-
b	625	0	39	58	3	82
48 a	8	-	-	-	-	-
b	28	10	80	0	60	15
c	18	0	0	0	100	-35
49 a	4	-	-	-	-	-
b	92	0	28	62	13	12
50 a	2	-	-	-	-	-
b	250	5	26	69	15	67
51 a	3	-	-	-	-	-
b	88	0	53	38	9	21
55 a	4	-	-	-	-	-
b	125	0	20	56	14	-68
55 a	41	0	0	100	0	-
b	120	0	0	91	9	-4
c	298	0	15	77	8	5

* Chemical forms separated by a modified Chang and Jackson procedure (9).
† "a" sample is check soil, "b" and "c" samples contain residual As from pesticide applications.

reduction in weight of 4-week-old corn seedlings. Figure 1 shows that 69 μg As/gram of soil (ppm) from Al-As, 77 ppm As from Ca-As, and 325 ppm As from Fe-As gave the same reduction in growth (50%, GR_{50}) as 42 ppm As from NaH_2AsO_4. This indicates that at this particular point, $Al(H_2AsO_4)_3$ is 62% as toxic, $Ca(H_2AsO_4)_2$ is 54%, and $Fe(H_2AsO_4)_3$ is 13% as toxic as the $Na(H_2AsO_4)$. It was necessary to determine the $Fe(H_2AsO_4)_3$ value by extrapolation. Arsenic found in each fraction of the contaminated soils was multiplied by the GR_{50} percentage value for that fraction and these modified As fractions were then summed to give an available As value.

Pure compounds were subjected to the modified P fractionation procedure after addition to Lakeland loamy sand. Although some As was found in all fractions, the major portion (> 75%) of the As was found in the fraction corresponding to the added As compound. Total arsenic and the easily soluble-, Fe-, Al-, and Ca-arsenates found in the soils are presented in Table 2. The "a" samples are check soils, whereas the "b" and "c" samples (Table 1 and 2) are two different samples from the same contaminated field. Table 1 contains a list of soil properties which

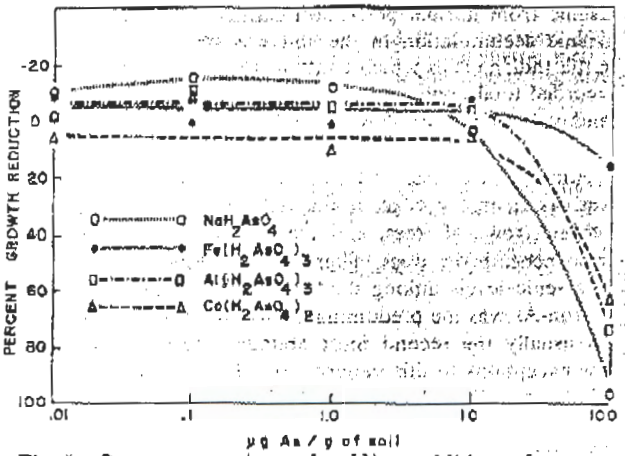

Fig. 1—Corn response (4 weeks old) to additions of arsenate salts to a Lakeland loamy sand.

were determined. All of the Mississippi and Alabama soils were experimentally contaminated in the field (1932 and 1962, respectively) with As. All other soils received the

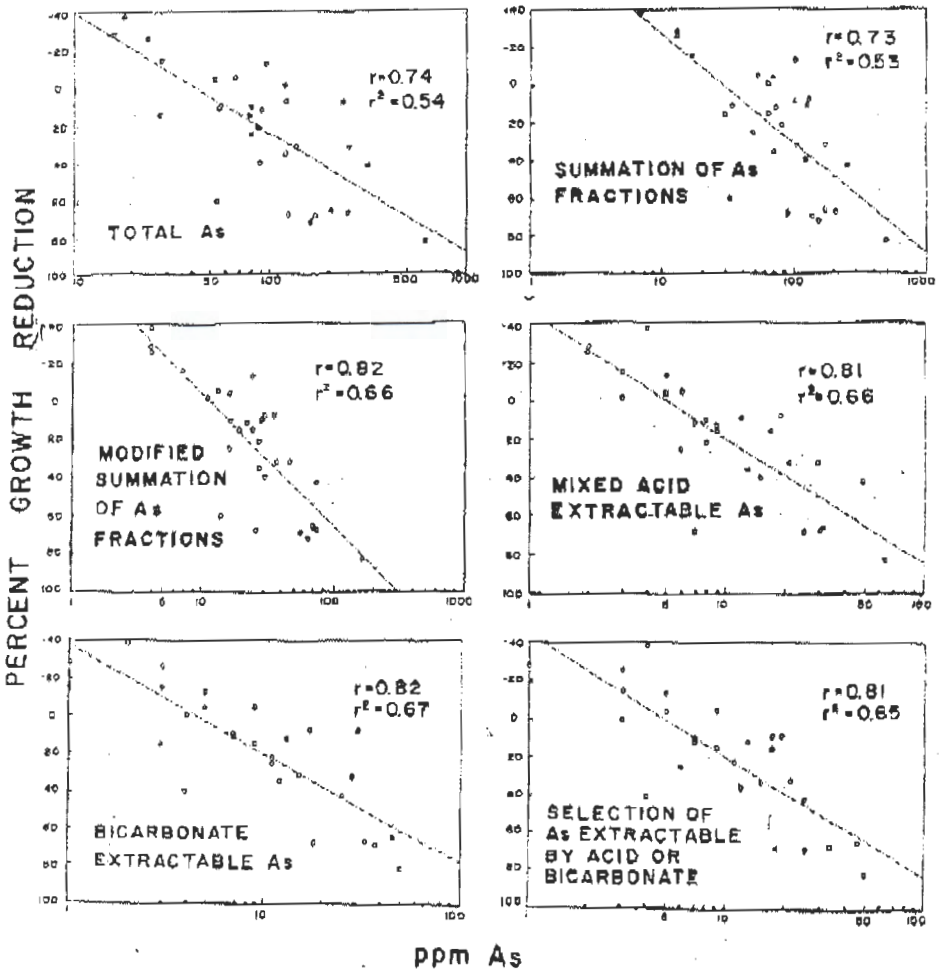

Fig. 2—A comparison of six methods for the estimation of As availability by a correlation between growth reduction (4-week-old corn) and the log of As concentration in soils.

02/14/2001 09:07 5054243202 JILL PODOLSKY, EHA PAGE 08
01/08/2001 14:03 8 CTR POP HEALTH PAGE 04

arsenic from normal pesticide treatment. An indication of arsenic accumulation in the soil was observed (Table 2) in the Indiana soils which contained more than 90% of the recorded total application in the surface 15 cm. The Maryland sample (55 c) contained 71% of the estimated As application. Sampling site is important in observing the accumulation of As. The Maryland check sample (55 a) was taken on the edge of the field, the "b" sample from between rows of trees, and the "c" sample was obtained from beneath the trees. There is a corresponding increase in arsenic levels among the samples (a to c).

Iron-As was the predominant form in most soils with Al-As usually the second most abundant form. However, a few exceptions to this pattern exist. In some soils (soil no. 14, 16, 52) the Al form was highest in abundance and in two soils (soil no. 48 b and 48 c) Ca-As was predominant. The high reactive Al or Ca contents of these soils (Table 1) could explain this change. Also the reactive Fe content was lower than the reactive Fe content in most of the soils tested. All soils exhibiting a growth reduction in corn greater than 60% contained measurable amounts of "Easily Soluble" As with one exception (soil 42 from New York). All of the treated Idaho soils and one soil each from Florida and Indiana contained detectable amounts of "Easily Soluble" As.

Correlations between the log of the different test values and the reduction in plant growth caused by As are presented in Fig. 2. All correlations are significant at the 1% level.

Applying an availability factor to the amount of As present in each form and summing these amounts to give total-available As yields a better correlation than the summation of As fractions ($r = 0.82$ vs 0.73, respectively). This reinforces the hypothesis that the individual chemical As species do have different availabilities and, as a result, phytotoxicities.

Correlations between the log of As extracted mixed acid ($0.025N$ H_2SO_4 and $0.05N$ HCl) and $0.5N$ $NaHCO_3$ and plant growth indicated that both methods were nearly equal ($r = 0.81$ and 0.82, respectively). The mixed method is used routinely to test for available P in weathered acid soils of the Eastern United States. The bicarbonate procedure is used to test for available P in the western alkaline soils. These two methods were selected since the soils used in this study came from both regions. Two other tests, the "modified summation of As fractions" and the combination of mixed acid method for soils with pH < 6.5 or the bicarbonate method for soils pH ≥ 6.5 also gave correlation coefficients of 0.82 and 0.81, respectively, with plant growth. Correlations between plant growth and total As, as well as the "summation of As fractions," were not as good as with the other four techniques ($r = 0.74$ and 0.73, respectively). The four best correlations account for 66–67% of the variation in plant growth (Fig. 2). Some of the remaining variation might be accounted for by difference in the soil fertility of the two samples which were obtained from each location.

The calculated regression for all methods of determining available As indicate that at the lower As levels (40–50 ppm As for total and the summations of As fractions, 5 ppm As by extraction) a positive growth response is predicted. This positive response is real and has been observed many times by the authors in their greenhouse work. This response is presumably due to a stimulation of the plant systems by small amounts of As since other pesticides (e.g., 2, 4-D) stimulate plant growth at sublethal dose levels.

The four best techniques for estimating As availability to plants resulted in correlation coefficients of 0.81 to 0.82. Of these four techniques, the $0.5N$ $NaHCO_3$ method or the $0.05N$ HCl and $0.025N$ H_2SO_4 mixed acid method were the least laborious, and may be preferred for determining phytotoxic (available) As.

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A Comparison of Analytical Methods for Soil Zinc[1]

A. L. Brown, James Quick, and J. L. Eddings[2]

ABSTRACT

Soils from 92 fields in California were analyzed for extractable Zn by four methods, namely, ammonium acetate-diphenylthiocarbazone (dithizone), diethylenetriaminepentaacetic acid (DTPA), 0.1*N* HCl, and disodium ethylenediaminetetraacetate (Na_2EDTA). Each soil was tested in the greenhouse for plant response in relation to Zn applications. All four methods exhibited a high degree of correlation with each other; the highest correlation coefficient was obtained for the comparison of dithizone with DTPA.

Calculated predictive values for DTPA, dithizone, 0.1*N* HCl, and Na_2EDTA were 83, 79, 73, and 72%, respectively, for the 92 soils studied. On this basis the DTPA is preferable to the others and the critical level is approximately 0.5 ppm Zn. For some soils, more than one method may be required to properly characterize the Zn status. The choice of method will depend upon the correlation with plant response, equipment and facilities available, and to some extent on the preference of the analyst.

Additional Key Words for Indexing: dithizone, diethylenetriaminepentaacetic acid, sodium triaminetetraacetate, hydrochloric acid.

Several analytical methods have been used to diagnose Zn deficiency in soils. The two most common procedures utilize extraction with 0.1*N* HCl or ammonium acetate-diphenylthiocarbazone (dithizone). Extraction with disodium ethylenediaminetetraacetate (Na_2EDTA) has also been done, but less correlative data are available for this than for the other two methods. More recently extraction with diethylenetriaminepentaacetic acid (DTPA) has been proposed (Lindsay and Norvell, 1969)[3] as a satisfactory method for Zn and Fe deficiency diagnosis. The methods are not equally rapid or equally adapted to routine analysis. The choice of procedure may depend on the availability of suitable equipment, even though relationships with plant growth may be similar.

The dithizone extraction method has been shown to be a useful method for diagnosing Zn deficient soils (Brown and Krantz, 1961; and Brown, Krantz, and Martin, 1962). This provides an excellent basis on which to compare other methods of analysis.

MATERIALS AND METHODS

Soil samples from 92 fields in California were analyzed for extractable Zn by four methods:

1) The Zn was determined colorimetrically using the ammonium acetate-dithizone method (Brown and Krantz, 1961; and Shaw and Dean, 1952).

2) 0.1*N* HCl-extractable Zn and titratable alkalinity were determined essentially as outlined by Nelson, Boawn, and Viets (1959); but the extractant, after being made to volume, was run through an anion exchange resin column (Johnson and Ulrich, 1959) to separate the Zn from other heavy metals.

3) DTPA-extractable Zn was determined according to the method of Lindsay and Norvell (1969).[3]

4) To determine Na_2EDTA-extractable Zn, 2 g of air-dry soil was shaken in 20 ml of 1% aqueous Na_2EDTA solution for 2 hr. The suspension was filtered through acid-washed, fluted filter paper. Zinc in the filtrate was determined by atomic absorption spectrophotometry (Allan, 1961).

Response to soil-applied $ZnSO_4$ was determined by greenhouse pot tests using sweet corn (*Zea mays* L. 'Golden Cross Bantam T51'). Treatments were usually in duplicate or triplicate. Response to treatment is designated to be a statistically significant ($\alpha = 0.05$) increase in dry weight of plant material.

Additional analyses were as follows: organic carbon was determined by dry combustion; total Zn by X-ray fluorescence (Whittig, Buchanan, and Brown, 1960). Determination of $CaCO_3$ was made manometrically (Williams, 1949), and the pH of a saturated paste was determined with a pH meter.

Correlation coefficients (Table 1) were calculated for carbon, $CaCO_3$ equivalent, and total Zn, compared to extractable Zn values for each of the four methods. It was considered unnecessary to include the data on the various chemical properties of the soils. The total Zn for the 92 soils ranged from 28 to 136 ppm, with an average was 86 ppm. Correlations for pH as compared to other properties and extractable Zn were not calculated since most soils were between 7.0 and 8.2.

[1] Contribution from Dept. of Soils & Plant Nutrition, University of California, Davis. Received Mar. 9, 1970. Approved Sept. 15, 1970.

[2] Lecturer in Soils, Agriculturist, and former Laboratory Technician, respectively.

[3] Lindsay, W. L., and W. A. Norvell. 1969. A new DTPA-TEA soil test for Zn and Fe. Agron. Abstr. p. 84.

02/14/2001 09:07 5054243202 JILL PODOLSKY, EHA PAGE 10
01/08/2001 14:03 8 CTR POP HEALTH PAGE 06