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COMPARISON OF FOUR EXTRACTION METHODS FOR THE ESTIMATION OF AVAILABLE ZINC FOR WHEAT

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Abstract. All the four methods of Zn extraction i.e. DPTA (diethylenetriaminepentaacetic acid), EDTA (ethylenediaminetetraacetic acid), HCl, and NH_4OAc successfully measured plant-available Zn and predicted its deficiency for wheat crop. The correlation coefficients of various methods with Zn concentration in plants were 0.91, 0.86, 0.87, and 0.70 and with total Zn contents 0.87, 0.86, 0.82, and 0.56 respectively. The critical Zn concentration of four methods that distinguished Zn-deficient from nondeficient soils were 0.34, 0.45, 2.4 and 0.48 ppm respectively. The DTPA method is superior to other methods for the current calcareous soils. It is recommended as a routine method as it also simultaneously extracts plant available Cu, Fe and Mn contents of soils.

Soil tests have played an importanat role in Zn fertilization of several crops in many parts of the world. For example, the DTPA extractable Zn showed significant correlation with that taken up by corn[3,6], sorghum, potato, beans, and asparagus [2] and predicted Zn deficiency in thier plants. Similarly other chemical reagents such as dithizone [10], HCl and EDTA[11] have successfully been used to extract available Zn for several crops. The efficiency of various tests, however, varied highly with types of soils and plant species [12]. Zinc extraction methods have never, so far, been evaluated on alkaline calcareous soils of Pakistan and for local plant species. The present studies compare four chemical methods for predicting Zn avaiablility to wheat.

Materials and Methods

Soils Used and Methods of Zn Extraction

Twenty-one surface soils of Gujranwala, Sheikhupura, Lahore and Faisalabad districts of the Punjab were used for these experiments. The soils were air-dried, crushed in a wooden mortar to pass through a 2-mm sieve and analysed for various physicochemical characteristics. In general, the soils were clayey in texture, highly alkaline, moderately calcareous, rich in soluble salts and contained low contents of organic matter (Table 1).

Available Zn in the air-dried soil samples was determined by the following methods:

(a) *EDTA Method.* The extraction solution contained 0.01M EDTA and 1.0M $(NH_4)_2CO_3$ at pH 8.6. Ten g soil was shaken with 20 ml solution for 30 min and Zn in the filtrate was determined with atomic absorption spectrophotometer [11].

(b) *DTPA Method.* The extraction solution contained 0.005M DTPA, 0.1M TEA (triethanolamine) and 0.01M $CaCl_2$ at pH 7.3. Ten g soil was shaken with 30 ml solution for 2 hr. Zinc in the filtrate was determined with atomic absorption spectrophotometer [8].

(c) *HCl Method.* Two g soil was mixed with 50 ml 0.1M HCl solution. After standing overnight the mixture was shaken for 30 min. Zinc in the filtrate was determined with atomic absorption spectrophotometer.

(d) *NH_4OAc Method.* Five g soil was shaken with 20 ml of 1.0M NH_4OAc at pH 4.8 for 1 hr [4]. Zinc in the filtrate was determined by atomic absorption spectroscopy.

Studies on Response of Wheat to Zn Fertilization

Subsamples of various soils at 3.7 kg were filled in polythene-lined plastic pots. The basal fertilizer dressings consisted of 75 ppm N as urea and 13 ppm P as KH_2PO_4. Zinc (as zinc sulphate) was applied at 5 ppm. Each treatment with a control was replicated thrice. All the fertilizers were well mixed throughout the pots before sowing the plants. Twelve

TABLE 1. RANGES AND MEANS OF CHARACTERISTICS OF 21 SOILS USED FOR WHEAT EXPERIMENTS.

Soil characteristics*	Ranges and means of characteristics	
	Range	Mean
Texture	Clay loam–clay	
pH	9·42–7·55	8·38
Organic matter (%)	1·25–0·53	0·89
$NaHCO_3$ extractable P (ppm)	25·80–1·00	7·79
DTPA extractable Zn (ppm)	1·88–0·32	0·69
$CaCO_3$ equivalent (%)	4·52–0·12	1·26
HCO_3 (meq/l)	5·00–2·30	3·60
Ca+Mg (meq/l)	3·80–0·10	2·56
EC (mmhos/cm)	4·20–058	0·99

*$CaCO_3$ was determined by HCl neutralization, organic matter by dichromate oxidation, and pH, EC, Ca+Mg and HCO_3 contents in 1:2 soil–water extract.

* Now at Arab Development Institute, P.O. Box 8004, Tripoli, Libya.

TABLE 2. CONCENTRATION AND TOTAL ZN CONTENTS IN WHEAT PLANTS AND THAT EXTRACTED BY VARIOUS METHODS FROM 21 SOILS.

Soil No.	Zn uptake by wheat		Zn extracted by four methods			
	Zn concn in plants (ppm)	Total Zn contents in plants (μg/pot)	EDTA (ppm)	DTPA (ppm)	NH_4OAc (ppm)	HCl (ppm)
31	34·03	239·21	1·95	1·56	2·28	10·7
32	25·66	156·54	0·80	0·50	0·72	6·5
33	24·80	174·01	1·30	0·86	0·64	11·0
34	40·94	288·92	2·25	1·88	1·20	13·0
35	18·17	115·12	0·70	0·56	0·76	30·5
36	17·01	59·51	0·70	0·40	0·52	2·8
37	20·11	188·95	0·70	0·66	0·64	4·7
39	26·81	180·12	1·05	0·66	0·64	4·2
40	10·38	76·34	0·25	0·32	0·28	1·7
41	14·99	83·84	0·45	0·42	0·64	2·4
42	17·01	119·35	0·60	0·56	0·72	2·9
43	23·36	101·90	1·10	0·60	0·52	3·1
44	15·57	66·66	1·00	0·46	0·48	3·1
45	29·13	157·89	1·10	1·28	1·36	10·5
46a	17·88	138·62	0·96	0·52	0·28	3·2
46b	26·24	176·91	1·55	0·96	0·48	4·2
47	23·36	145·33	0·80	0·50	0·48	5·6
48	20·47	153·37	1·10	0·56	0·52	4·0
49	13·63	96·65	0·45	0·34	0·48	1·9
50	18·17	117·53	0·70	0·56	0·52	4·6
51	14·56	102·19	0·45	0·34	0·32	2·4

seeds of Chenab-70 wheat were sown in each pot in November 1973. The seedlings were thinned to 8 per pot a week later. The soils were maintained at field capacity by daily addition of deionized water throughout plant growth.

Wheat seedlings were harvested about 15 days before flower initiation. They were rinsed in deionized water, dried and ground in a Wiley mill fitted with stainless steel blades and other interior parts of the grinding chamber; 1 g portions of ground plant material were digested with 25 ml diacid mixture (redistilled HNO_3 and $HClO_4$ at 4:1) and Zn in the diluted digest was determined by atomic absorption spectrosocpy [1]. Correlation coefficients between soil-extractable and plant Zn were calculated using standard statistical procedures.

Results and Discussion

Relationship of Zn Uptake by Wheat with Soil Zn Extracted by Four Methods

TABLE 3. CORRELATION BETWEEN Zn UPTAKE BY WHEAT AND THAT EXTRACTED BY FOUR METHODS FROM 21 SOILS.

Method of Zn extraction	Correlation coefficient* with	
	Zn concn in plants	Total Zn contents in plants
EDTA	0·86	0·86
DTPA	0·91	0·87
HCl	0·87	0·82
NH_4OAc	0·70	0·56

*All the correlation coefficients of concentration and total Zn contents are significant at $P<0·01$.

The amount of Zn uptake by wheat and that extracted by four methods from 21 soils are shown in Table 2. The DTPA, EDTA, HCl and NH_4OAc extractable Zn varied from 0.32 to 1.88, 0.25 to 2.25, 1.70 to 13.0, and 0.28 to 2.28 ppm respectivley from various soils. These amounts are almost identical to those extracted by these methods from alkaline calcareous soils of Colorado [11] and California. The amount of Zn extracted by the four extractants ranged in the order of HCl > EDTA > DTPA = NH_4OAc. Similar order of their efficiency was reported by earlier investigators [3,6,11] in alkaline soils of other regions.

All the four methods appeared to successfully measure available Zn for wheat plants. The DTPA method showed the highest correlation coefficients of 0.91 and 0.87 ($P \angle 0.01$, Table 3) with concentration and total contents of Zn in plants respectively. The EDTA and HCl methods were slightly inferior but quite effective in extracting plant available Zn. Their correlation coefficients with concentration and total Zn contents in plants were 0.86, 0.87 ($P \angle 0.01$) and 0.86, 0.82 ($P \angle 0.01$) respectivley. The NH_4-OAc method was much inferior to the other methods showing correlation coefficient of only 0.70 with concentration and 0.56 with total Zn contents in wheat plants.

Many investigators have compared these methods for predicting available Zn for various plant species and found their superiority to highly depend upon the nature of soils used. The DTPA method was shown to be superior to other methods on alkaline calcareous soils [3,12] and is being used successfully in predicting Zn deficiency for several crops on alkaline soils of Colorado, Washington and California [2,3,5]. The soils of the present studies resemble those of Colorado and California in many physicochemical properties and DTPA method appears also superior for these soils. This method seems to have added advantage for the soils of the current studies since it was also found superior for Cu determination[7] and thus both Zn and Cu can be determined from the same extract. Earlier researchers reported it also to predict Fe and Mn availability to crops on calcareous soils [12].

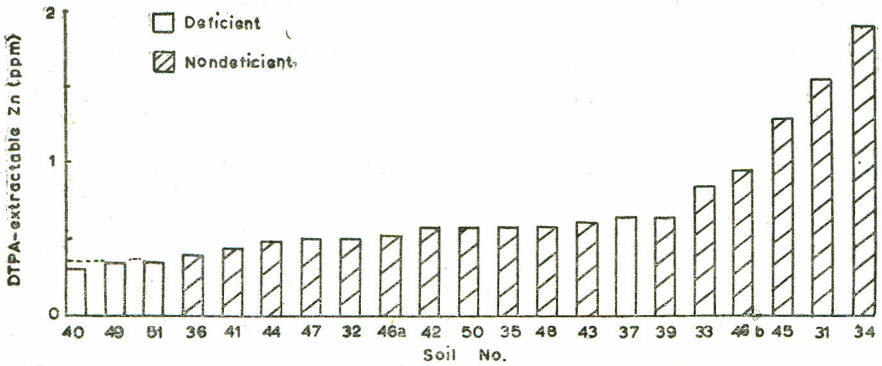

Fig. 1. Response of wheat to soil-applied $ZnSO_4$ in relation to DTPA-extractable Zn on 21 soils.

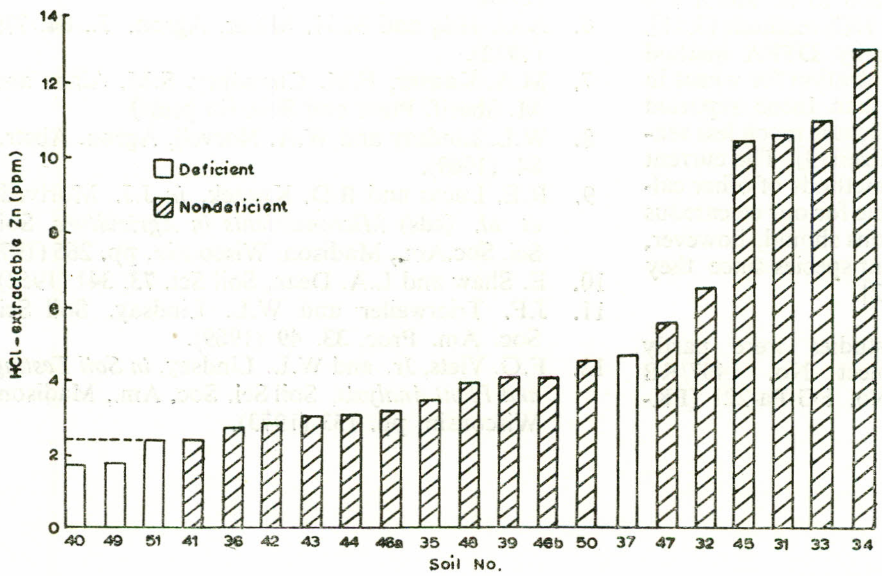

Fig. 2. Response of wheat to soil-applied $ZnSO_4$ in relation to HCl-extractable Zn on 21 soils.

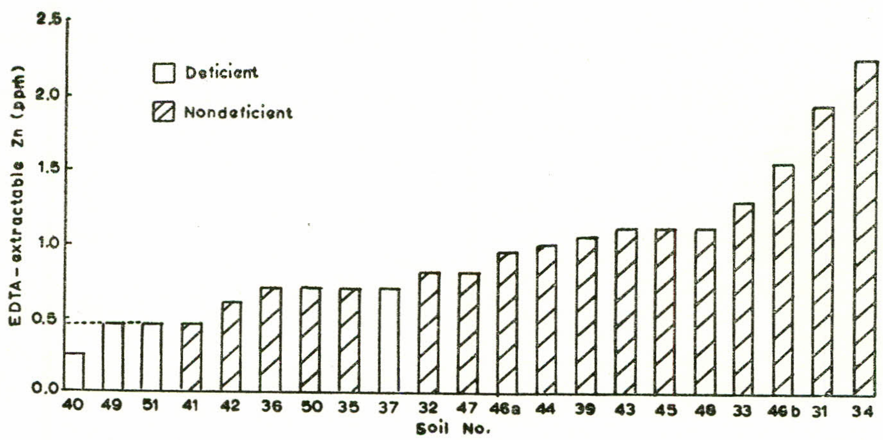

Fig. 3. Response of wheat to soil-applied $ZnSO_4$ in relation to EDTA-extractable Zn on 21 soils.

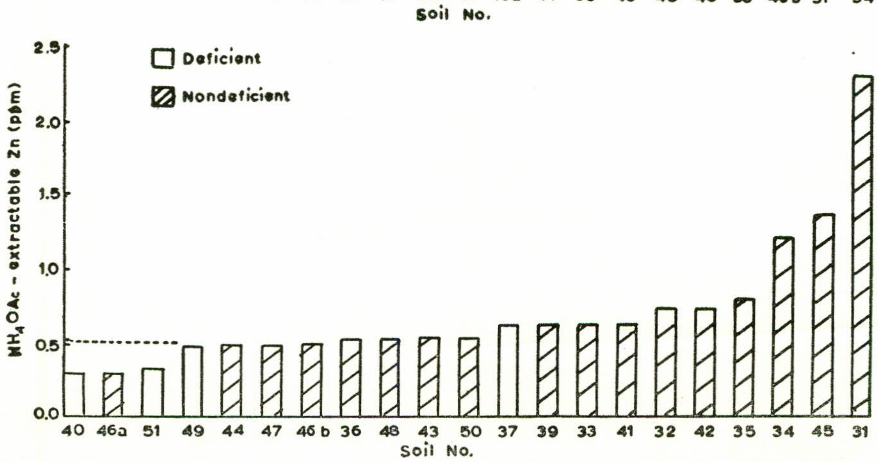

Fig. 4. Response of wheat to soil-applied $ZnSO_4$ in relationt o NH_4OAc-extractable Zn on 21 soils.

Critical Level of Extractable Soil Zn for Wheat

A successful soil test should distinguish soils that respond to Zn fertilizer from those that do not. Various Zn extraction methods were put to this test. When extractable Zn was plotted against plant response ($P \angle 0.05$) to added fertilizer (Figs. 1 to 4), all the methods clearly separated Zn deficient from nondeficient soils. About 0.34 ppm DTPA, 2.4 ppm HCl and 0.45 ppm EDTA extractable Zn appeared to be critical levels. The critical level of NH_4OAc Zn was not very distinct but could approximately be taken as 0.52 ppm. Other researchers determined critical Zn levels for various Zn sensitive crops such as corn and found them to be about 1.4 ppm by EDTA and 5.0 ppm by HCl methods [3,11]. It ranged from 0.5 to 0.8 ppm by DTPA method [3,8,12]. The critical Zn concentration for wheat in the present experiments is less than those reported for corn by these workers. But wheat is much less sensitive than corn to added Zn fertilizers[9]. The current results indicate soil extraction methods of other calcareous region to be equally suitable for our calcareous soils. Critical soil Zn concentrations should, however, be evaluated for various local plant species since they strongly vary with crop types[12].

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