

MICRONUTRIENT CONCENTRATIONS IN CORN AND A COMPARISON OF THE VARIOUS EXTRACTANTS FOR SOIL Zn AND Cu

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Leaf survey for micronutrient levels in corn indicated no deficiency of Zn, Cu, Fe and Mn in the various soils despite the latter, having found responsive to the micronutrient fertilization in wheat, expected to be deficient in the different micronutrients.

Corn (var: Neelum) was grown for 33 days with 10 ppm of Zn and Cu in pots containing 4.5 kg each of the 23 soils taken from those areas to know their response and suitability of some extractants for measuring available Zn and Cu in soils. Dry weight results showed that corn was not that much responsive to Cu as to Zn. Concentration of Zn in plants, grown without Zn, ranged from 13–15 ppm which increased to 2 to 3 times with Zn application while that of Cu, with or without Cu application, varied from 8–14 ppm falling within the adequate range (4–20 ppm) of Cu. Both showed a depressive effect on the uptake of each other.

From Zn and Cu contents extracted by various extractants, the soils were generally noticed low in Zn but not in Cu. Correlation studied between soil Zn extracted by various extractants and plant Zn uptake was highly significant ($r=0.76-0.88$) and that between soil Cu and plant Cu uptake was very poor. Soil available-Zn and plant dry weights had non-significant negative correlation.

INTRODUCTION

Corn (*Zea mays L.*) is used as a staple food after wheat in certain parts of the country. Besides fodder it forms an important component of the various household products including feeds available in the local market. It is grown on a large area in the country. It generally follows wheat and occasionally rice in crop rotation. Among the factors affecting growth of cereal crops, deficiency of micronutrients particularly that of Zn and to some extent Cu is turning out to be a serious problem in rice and wheat in the region [1–4]. Though the genetic variability in plant species plays an important role in their susceptibility to the deficiency of micronutrients [4], corn may be expected to be affected with their deficiency specially that of Zn and Cu. However, very little is known about Zn and Cu nutrition of corn in the local soils.

The present investigations were, therefore, carried out to assess Zn and Cu status of corn plants in the fields and to study their response as well as the suitability of various extractants for the determination of their available amounts in a number of soils grown to corn in pots.

MATERIALS AND METHODS

1. *Field Experiment.* Leaf samples of corn plants compris-

ing various varieties were collected at about silking stage from various locations on the farmers' fields in the corn growing areas. After washing with distilled water, the leaf samples were dried at 65° in an oven before grinding to a 20 mesh powder. The plant materials were digested in $\text{HNO}_3\text{-HClO}_4$ mixture and the concentrations of Zn, Cu, Fe and Mn were determined on atomic absorption spectrophotometer.

2. *Pot Experiment.* Four and a half kilograms of each of the 23 soils were placed in polyethylene-lined plastic pots. The physico-chemical characteristics of the soils are shown in Table I. Sand, silt, and clay contents in the soils were determined by Bouyoucos hydrometer, pH in 1:1; soil/water suspension on the pH-meter using a glass electrode and electrical conductivity on the conductivity meter as explained by Jackson [5]. Carbonate (CaCO_3) contents in the soils were measured with Puri's method as given by Piper [6]. Urea at the rate equivalent of 150 kg N and KH_2PO_4 equivalent of 90 kg P_2O_5 /ha were applied as a basal dose. Zinc and Cu were applied as their sulphates at 20 kg/ha each. All the fertilizers were added in the form of solution. The treatments were replicated three times in a completely randomized design.

Ten seeds of corn (variety: Neelum) were sown in each pot and the soils were maintained at field capacity during the growth period. Thirty three days after sowing, the plant

tops were harvested (the roots could not be separated from the soils), washed with deionized water and dried at 65° in an oven. The samples were ground to a powder in a Wiley micro-mill, fitted with stainless steel blades and sieve, and digested in HNO₃-HClO₄ mixture. Zinc and Cu concentrations in the plant digests as well as in the soil extracts obtained by DTPA [7], EDTA-(NH₄)₂CO₃ [8], NH₄OAc [9] and EDTA-Na [10] methods were determined on the atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

1. Field Samples.

Leaf Analysis. Leaf samples of corn plants, comprising of various varieties, were collected from the various farmers' fields in the corn growing areas and analyzed for Zn, Cu, Fe and Mn. Data so obtained were used to assess soils under corn for their micronutrient status. On the basis of the concentrations of ions in the leaves (Table 2) all soils could be considered to contain adequate supplies of Zn, Cu, Fe and Mn although the soils are known to have been given no micronutrient fertilizers in the past. However, analysis of soil samples collected later from different locations in the same areas, generally grown to corn and wheat, showed soils to be deficient in Zn but not in Cu (Tables 6 and 7) according to the critical limits of Zn and Cu reported for the soils [7, 9, 11]. Studies were, therefore, conducted to examine the response of corn to applied Zn and Cu in these soils in pots and to compare the various extractants for their usefulness in determining available Zn and Cu.

2. Pot Experiment

(a) Plant Growth and Ion Content. (i) Effect of Zinc: Application of Zn significantly increased the concentration (P=0.01) as well as the uptake (P=0.01) of Zn in the aerial portions of corn plants on all soils compared with the concentration and uptake of Zn in plants given no Zn fertilizer (Table 3). The concentrations of Zn in the tops fell, however, within the range of concentration that was usual for most crop plants including corn [11-13]. However, the concentration of Zn in the tops of most of the corn plants on soils given no Zn fell in the range of 13-15 ppm. Only a few soils which were also responsive, however, gave Zn concentration in plants up to 28ppm—a result which could be fortuitous. On the basis of the critical concentration of Zn reported for corn [11,14], a large number of the corn plants in the present experiment could be considered deficient in Zn.

Zinc application significantly (P=0.01) increased dry weights of plants, in general, on a large number of soils. In a few cases, however, there was little or no response while in others the dry weights decreased. While the pH of original soils approached to about 8.0, the other soil characteristics i.e. CaCO₃, electrical conductivity and the clay contents, differed from each other to varying degrees

Table 1. Ranges and average values of some of the physico-chemical properties of the soils used for the pot experiment.

Soil properties studied	Ranges	Average
Clay, %	8.8 -26.6	14.5
Silt, %	4.2 -40.0	24.3
Sand, %	41.0 -86.2	61.1
pH (1:1)	8.02- 8.70	8.16
Ec x 10 ³ , mmhos/cm	1.42- 6.25	2.67
CaCO ₃ , %	2.4 - 9.8	6.1
Organic matter, %	0.24- 1.31	0.79
DTPA-Zn, ppm	0.17- 1.10	0.36
DTPA-Cu, ppm	0.43- 2.37	1.16
DTPA-Fe, ppm	1.9 - 9.0	4.2
DTPA-Mn, ppm	5.5 -19.5	8.7

Table 2. Zinc, copper, iron and manganese contents in corn leaves sampled from various fields in the corn areas.

Site No.	Zn	Micronutrients studied		
		Cu	Fe	Mn
concentration, ppm				
1.	24	10	360	60
2.	21	9	240	55
3.	26	12	340	65
4.	26	14	260	70
5.	31	13	370	50
6.	39	11	650	70
7.	28	10	600	60
8.	24	17	425	100
9.	45	13	600	80
10.	27	13	*	170
11.	35	13	500	50
12.	33	13	400	80
13.	35	11	360	60
14.	31	10	185	50

*very high

amongst the soils and a causal relationship of any of these factors with ionic concentrations in the leaves or with the dry weights of plants could not be established. Increased Zn uptake, dependent on yield and concentration, with added Zn indicated the ability of soils to supply Zn as well as the capacity of corn to absorb and retain it to quite a high level without exhibiting toxic effects.

(ii) Effect of Copper. Application of Cu significantly (P=0.01) decreased plant growth on a number of soils (Table 4). On some soils, however, there was little or no response while on the others the dry weights increased.

Table 3. Effect of Zn application on growth and Zn uptake in corn grown on 23 soils in a pot experiment

Soil sites	*Zn applied, kg/ha					
	0		20		20	
	DMY g/pot	Zn conc	Zn ppm	Zn ppm	Zn µg/pot	µg/pot take
1. Jhang, AgriFarm.	9.0	8.0	21.3	46.3	192	370
2. Chimranwali Chak 54	4.4	8.8	13.0	38.7	58	342
3. Thekriwala, Wali Farm.	3.0	5.0	13.0	37.0	39	184
4. Gojra, Seed Farm	5.8	5.6	13.8	37.7	80	212
5. Toba Tek Sing Alharpind	5.3	5.8	15.5	32.7	82	189
6. Rejana, Ismail Farm.	6.3	5.9	15.3	42.3	98	250
7. Rejana, Chak 285/J.B.	3.6	4.7	12.0	34.2	44	162
8. Kamalia, Chaddar	6.1	8.7	14.7	33.0	90	220
9. Chichawatni, Chak-17/11-L	7.8	9.1	14.7	36.0	115	329
10. Burewala, Zaheer Farm.	4.1	7.2	13.8	38.2	57	274
11. Chauki Chamb, Chak-66	3.5	5.7	11.2	35.5	39	203
12. Burewala, Textile Mills	7.5	9.1	13.2	35.0	98	320
13. Arifwala, Agri. Farm.	6.2	6.9	13.2	47.7	82	330
14. Lahorianwala Chak-7/11-L	7.1	8.9	15.5	34.8	110	311
15. Multan, Qadirpur	8.3	12.2	15.5	26.3	127	320
16. Khanewal, Musa Virk	6.4	7.1	13.0	49.5	83	349
17. Sahiwal, Chak 187/9-L	4.1	6.5	16.2	47.2	67	307
18. Sahiwal, Naseer Farm	4.6	6.0	13.8	43.5	64	263
19. Yousafwala, Maize Farm	7.2	8.6	13.7	27.3	99	234
20. Mian Channu, Mamdot Farm	9.8	12.1	14.7	33.0	144	401
21. Kabirwala, Nawaz Farm	6.0	5.6	12.2	54.0	73	301
22. Khanewal, Kacha Khoo	6.3	5.8	13.8	38.8	87	227

23. Jhang, Iftikhar Farm	6.8	8.7	28.8	34.8	197	198
LSD	P(0.05)		0.32	1.30	10.85	
	P(0.01)		0.43	1.71	14.33	

*DMY—dry matter yield of corn tops, conc—concentration, LSD—least significant difference, P_(0.05)—significant at 5 % level of probability and P_(0.01)—significant at 1 % level of probability; LSD for soils with regard to DMY is 0.90 and 1.18, to conc as 3.59 and 4.75 and to uptake as 30.03 and 39.88 at 5 % and 1 % level of probability respectively.

Copper concentration in the corn tops, both where Cu was or was not applied to soils, ranged from 8–14 ppm; which are much higher than the concentration (4 ppm) where deficiency was likely to occur and are below the limit (20 ppm) where toxicity could be expected[11]. It is not, therefore, possible to relate the growth effects to Cu concentrations, which were neither low (deficient) nor high (toxic) in the plants grown with and without applied Cu. Differential response of soils (P=0.01) in respect of yield and concentration as well as uptake of Zn and Cu in corn to applied Zn and Cu could be related to soil variabilities[15,16].

(iii) Zinc-Copper Interaction: While it increased the growth of plants (Table 3), Zn decreased significantly (P=0.01) Cu concentration and uptake in the corn tops on a large number of soils (Table 5) due in part, at least, to "dilution" from the dry weight increase. On some soil types Zn did decrease both the uptake as well as the plant concentrations of Cu though it decreased the dry weights of tops—an effect which could be ascribed, in part, to the antagonistic effects of Zn on Cu uptake. Reports relevant to Zn-Cu interactions conflict with each other in that, in one case Zn had little effect on Cu absorption from its luxuriant supplies [17] while in another it increased its absorption[18]. Since Cu contents of roots are not available, the nature of the mechanism of Zn effect on Cu needs to be looked into further. On the contrary, Cu also decreased significantly (P=0.01) the concentrations as well as uptake of Zn in plants on various soils in this experiment. Notwithstanding the effects of Cu on Zn, the concentrations of Cu in plants themselves remained little affected. Copper uptake was, however, variable among plants from being suppressed (9 soils) to being enhanced (9 soils). The nature of the effects needs to be examined further since the soil characteristics studied in the present experiment (Table 1) could not be related to the growth effects or to the absorption effects obtained. Elsewhere, Cu has been shown to depress Zn absorption in barley roots[19,20] and translocation from roots to the tops in bean seedlings[21] from the short term solution cultures. Since it was beyond the scope of the present investigation to collect data on root Zn contents, the depressive effects of Cu on Zn

Continued

Table 4. Effect of Cu application on growth and Cu uptake in corn grown on 23 soils in a pot experiment

*Soil sites	Cu applied, kg/ha							
	0		20		0		20	
	DMY,	g/pot	Cu conc.	ppm	Cu uptake	µg/pot		
1. Jhang, Agri. Farm	9.0	8.1	9.0	8.5	81	69		
2. Chimranwali Chak-54	4.4	5.4	8.3	10.5	37	57		
3. Thekriwala, Wali Farm	3.0	3.7	9.2	11.5	27	43		
4. Gojra, Seed Farm	5.8	5.2	10.0	11.3	58	59		
5. Toba Tek Singh, Alharpind	5.3	5.7	8.3	9.7	44	55		
6. Rejana, Ismail Farm	6.3	5.8	11.3	10.5	72	61		
7. Rejana, Chak 285/J.B.	3.6	2.8	8.7	9.5	32	26		
8. Kamalia, Chaddar	6.1	7.2	14.0	14.2	85	101		
9. Chichawatni, Chak-17/11-L	7.8	7.5	9.5	9.2	75	69		
10. Burewala, Zaheer Farm	4.1	2.4	14.2	14.7	58	35		
11. Chauki Chamb, Chak-66	3.5	2.1	8.8	9.5	31	20		
12. Burewala Textile Mills	7.5	5.3	10.0	10.5	75	56		
13. Arifwala, Agri. Farm	6.2	5.1	9.0	7.7	56	39		
14. Lahorianwala Chak-7/11-L	7.1	8.5	12.5	11.5	89	98		
15. Multan, Qadirpur	8.3	8.6	12.0	13.3	100	114		
16. Khanewal, Musa Virk	6.4	6.6	9.5	9.7	61	83		
17. Sahiwal, Chak 187/9-L	4.1	4.8	9.5	10.0	39	48		
18. Sahiwal, Naseer Farm	4.6	3.7	10.0	10.0	46	37		
19. Yousafwala, Maize Farm	7.2	6.7	8.8	9.7	64	65		
20. Mian Channu, Mamdot Farm	9.8	10.3	8.7	9.2	85	94		
21. Kabirwala, Nawaz Farm	6.0	4.2	9.5	11.3	57	47		
22. Khanewal, Kacha Khoo	6.3	4.9	6.8	8.8	43	43		
23. Jhang,	6.8	5.6	6.8	8.8	47	49		
LSD	P(0.05)		0.32		0.37		4.01	
	P(0.01)		0.43		0.49		5.30	

*See Table 3 for description of sites and other legends. LSD for soils with regard to DMY as 0.90 and 1.18, to conc as 1.03 and 1.37 and to uptake as 11.10 and 14.66 at 5 % and 1 % level of probability respectively.

Table 5. Effect of Zn or Cu application on Cu or Zn uptake respectively in corn grown on 23 soils in a pot experiment.

*Soil sites	Zn applied, kg/ha				Cu applied, kg/ha			
	0 Cu conc ppm	20 Cu uptake	0 Cu uptake	20 µg/pot	0 Zn conc ppm	20 Zu up take	0 Zu up take	20 µg/pot
1. Jhang, Agri. Farm	9.0	8.3	81	66	21.3	16.0	192	129
2. Chimranwali, Chak-54	8.3	6.5	37	57	13.5	10.7	58	58
3. Thekriwala, Wali Farm	9.2	6.7	27	33	13.0	12.0	39	45
4. Gojra, Seed Farm	10.0	8.3	58	47	13.8	11.0	80	58
5. Toba Tek Singh, Alharpind	8.3	8.0	44	46	15.5	13.7	82	78
6. Rejana, Ismail Farm	11.3	8.0	72	47	15.3	11.2	97	65
7. Rejana, Chak 285/J.B.	8.7	6.8	32	32	12.0	7.8	44	22
8. Kamalia, Chaddar	14.0	7.3	85	67	14.7	10.7	90	76
9. Chichawatni, Chak-17/11-L	9.5	6.3	75	58	14.7	11.0	115	82
10. Burewala, Zaheer Farm	14.2	7.7	58	55	13.8	9.5	57	23
11. Chauki Chamb, Chak-66	8.8	8.0	31	46	11.2	—	39	—
12. Burewala, Textile Mills	10.0	8.3	75	76	13.2	11.2	98	59
13. Arifwala, Agri. Farm	9.0	6.0	56	42	13.2	12.2	82	62
14. Lahorianwala Chak-7/11-L	12.5	8.0	89	71	15.5	12.3	110	105
15. Multan, Qadirpur	12.0	6.8	100	83	15.3	12.8	127	110
16. Khanewal, Musa Virk	9.5	7.3	61	45	13.0	11.7	83	100
17. Sahiwal, Chak 187/9-L	9.5	8.8	39	58	16.2	10.8	67	51
18. Sahiwal, Naseer Farm	10.0	8.3	46	50	13.8	—	64	—
19. Yousafwala, Maize Farm	8.8	7.7	64	66	13.7	11.8	99	79
20. Mian Channu, Mamdot Farm	8.7	6.8	85	83	14.7	10.0	144	103
21. Kabirwala, Nawaz Farm	9.5	8.3	57	46	12.2	12.2	73	51
22. Khanewal, Kacha Khoo	6.8	7.3	43	43	13.8	15.5	87	76

table continued

23. Jhang, Iftikhar Farm	6.8	7.7	47	67	28.8	27.8	197	155
LSD $P_{(0.05)}$	0.37		4.01		1.30		10.85	
$P_{(0.01)}$	0.49		5.30		1.71		14.33	

*See Table 3 for description of sites and other legends.

Table 6. Zinc and Cu contents extracted by various extractants from 23 soils used for the pot experiment.

*Soil sites	DTPA	Extractants used				
		EDTA- (NH ₄) ₂ CO ₃ Zn conc., ppm	EDTA-Na	DTPA	NH ₄ OAc Cu conc., ppm	EDTA-Na
1.	0.53	2.20	1.60	2.70	1.20	2.70
2.	0.25	1.00	0.60	0.90	0.56	1.20
3.	0.28	1.26	0.70	1.40	0.56	2.10
4.	0.34	1.26	1.00	2.40	0.88	2.70
5.	0.34	1.20	1.00	2.10	0.72	2.60
6.	0.37	1.40	1.00	2.40	0.88	2.70
7.	0.23	0.86	0.50	0.80	0.64	1.30
8.	0.37	1.40	1.10	4.20	1.12	4.20
9.	0.31	1.36	0.80	1.00	0.56	1.20
10.	0.17	0.60	0.60	2.00	0.96	2.10
11.	0.23	0.80	0.70	0.74	0.64	1.10
12.	0.39	1.46	1.00	2.00	1.04	2.10
13.	0.23	1.14	0.70	1.40	0.72	2.00
14.	0.28	1.66	0.80	2.10	0.88	2.30
15.	0.34	1.40	1.10	2.70	0.88	3.20
16.	0.25	1.00	0.70	0.90	0.72	1.00
17.	0.25	1.18	1.00	1.40	0.88	1.90
18.	0.31	1.56	1.10	2.80	1.20	3.50
19.	0.51	2.02	1.20	2.90	0.88	3.40
20.	0.37	2.02	1.20	1.80	0.72	2.70
21.	0.37	1.72	1.30	2.20	0.72	3.30
22.	0.31	1.72	1.20	1.10	0.64	1.60
23.	1.10	4.20	2.90	3.40	1.58	3.80

*See Table for for description of sites.

noticed in this study could be difficult to relate either to its effect on absorption and/or translocation of Zn.

(b) *Soil Zn and Cu Extractable by Various Extractants.* In the present experiment, soil extracted with EDTA-NH₄CO₃ showed soils to contain Zn predominantly below 3 ppm (only soil No. 23 contained Zn about 4 ppm) and with DTPA to be about 0.5 ppm (Table 6) which in comparison to results obtained by others [7,22] represented low and critical levels of Zn in soils respectively. With EDTA-(NH₄)₂CO₃, Trierweiler and Lindsay [8] established a level of 1.4 ppm as the critical level. With a few exceptions, however, concentrations of Zn in most of

our soils approximated this value or fell below it.

Linear regressions calculated (Table 7) for Zn extractable by DTPA, EDTA-(NH₄)₂CO₃ and EDTA-NA and plant Zn concentrations ($r=0.88, 0.86$ and 0.88) and those for Zn uptake ($r=0.76, 0.80$ and 0.78) were highly significant, being in line with the suggestion that in calcareous soils these extractants, in comparison to others, were much more useful in predicting available Zn [8,12,23]. Some other studies [7,22,23] support also DTPA and EDTA as the more convenient and useful extractants for predicting available soil Zn. However, correlation between soil available-Zn values determined by various extractants and dry

Table 7. Correlation of Zn and Cu contents in soils extracted by various extractants with Zn content and its yield response as well as Cu contents of corn plants.

Extractants used	'r' values				
	Zn			Cu	
	Conc, in plants	Total content in plants	Yield response	conc, in plants	Total content in plants
DTPA	0.88**	0.76**	-0.12 ^{N.S.}	0.39	0.50
EDTA-Na	0.88**	0.78**	-0.18 ^{N.S.}	0.27	0.40
EDTA-(NH ₄) ₂ CO ₃	0.86**	0.80**	-0.15 ^{N.S.}	—	—
NH ₄ OAc	—	—	—	0.13	0.25

**Significant at P=0.01 and N.S.—not significant.

matter yield of corn tops was non-significant (Table 7).

However, since absorption data used in the correlation studies in the present experiment are based on plant responses on various soils to the only dose of Zn fertilizer, a more valid conclusion about the separation of deficient soils from the non-deficient ones could thus be made if separate studies on plant responses to varying doses of Zn fertilizer in relation to leaf symptoms on various soils are carried out under controlled conditions and the respective critical limits determined using Cate-Nelson procedures [11]. Based on this technique, a critical Zn level on a calcareous soils for sorghum was suggested to be 3 ppm elsewhere (EDTA-(NH₄)₂CO₃ extractable Zn). While the present studies showed almost all soils to be deficient in Zn as assessed by various extractants, the respective critical soil concentrations could not be suggested which could vary, among the various factors, with the kind of soils as well as the physico-chemical characteristics of the soils being studied. Thus with the extractants that gave the best assessment of critical limits under the conditions of the various studies on corn are known to range from 0.10 to 1.00 ppm [14,24].

Copper uptake versus soil Cu extractable by EDTA-Na gave 'r' value of 0.40 (Table 7) which is no better than the 'r' value of 0.57 [10]. Under the conditions of our experiment, only DTPA gave the best correlation of 0.50 for extractable Cu to that in the plants (Table 7). Apparently none of the extractants tested in the present experiment adequately measured the available soil Cu. Indeed, Cu concentrations in the plants were well above their deficiency limits whether Cu was applied or not. Under these conditions other soil factors might be considered in attempting to interpret the available data. For instance, the interaction of Cu with other elements such as Al and Fe in the soil is known to decrease Cu uptake by plants [15]. In one situation pH could be important [25] while in an other it is shown not to affect Cu uptake [10]. In addition, concentration of Zn could not also be important since the soil was

already in the deficiency range. In the present experiment, therefore, none of the above factors seems to be important since Cu concentrations in the plants were not low nor were the soils Cu-deficient, which is borne out by the soil tests that gave Cu by EDTA-Na from 1.00–4.20 ppm, by NH₄OAc from 0.56–1.68 ppm and by DTPA from 0.74–4.00 ppm (Table 6) exceeding its critical values of 0.5 ppm as determined by NH₄OAc [9] and of 0.4 ppm as determined by DTPA [26] methods.

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