Pakistan J. Sci. Ind. Res., Vol. 21, Nos. 5-6, October - December 1978

COMPARISON OF FOUR EXTRACTION METHODS FOR THE ESTIMATION OF AVAILABLE Cu FOR FLOODED RICE AND WHEAT

F.M. Chaudhry, M.A. Kausar, S.M. Alam and M. Sharif

Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan

(Received November 12, 1977; revised October 11, 1978)

The response of rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) to the application of $CuSO_4$ on several alkaline calcareous soils was investigated in the glass-house. The amounts of Cu extracted from the soils by four chemical methods were correlated with that taken up by the plants.

None of the four methods used could predict Cu availability for rice plants. The amount of Cu extracted by DTPA (diethylenetriamine pentaacetic acid) and NH_4OAc had little positive correlation either with concentration or total contents of Cu in plants. The HCl and EDTA methods significantly correlated soil-extractable Cu with plant Cu but the correlation coefficient (r) was small and, therefore, not useful as a soil test for Cu. None of these methods could distinguish Cu deficient from nondeficient soils.

The DTPA and NH_4OAc procedures adequately predicted Cu availability to wheat plants. The r value of both the methods with total Cu contents in plants was 0.70. The value improved to 0.84 when Cu concentration in plants was considered. The critical concentration of available soil Cu by the two methods is about 0.8 and 0.4 ppm respectively.

The chemical changes associated with flooding of rice soils which strongly affect Cu absorption by plants seem to be responsible for poor relationship between Cu extracted by various methods from dry soils and that taken up by submerged rice plants.

INTRODUCTION

Copper deficiency in flooded rice is quite prevalent on many alkaline calcareous soils [3]. Soil tests have played an important role in Cu fertilization of crops in several countries of the world. They have, however, been standardised mainly for upland crops. For example, DTPA extractable Cu shows significant correlation with that taken up by corn and other similar plants [11,14] and the method is being successfully used for predicting Cu availability for many plant species on calcareous soils of Colorado [20]. Similarly other chemical reagents such as EDTA [12,14], HCl[6] and NH_4OAc [7,8] have adequately measured Cu availability for several upland crops from various types of soils. Such tests have never been evaluated for lowland rice.

Rice is sown under submerged soil condition, which induces several chemical and electrochemical changes in its rhizosphere [15]. These changes such as increase in the concentration of H, P, Fe and Mn may profoundly affect Cu uptake by rice plants [5] rendering soil Cu tests on air-dry soils less reliable. The present studies were conducted to test this hypothesis. The results were compared with those of wheat grown on the same soils.

Table 1. Ranges and means of characteristics of several soils used for rice and wheat experiments.

Variable	Range	Mean
Texture	Clay loam–heavy clays	Clayey
pH*	9.4–7.6	8.4
Organic matter (%)	1.2–0.6	0.9
$NaHCO_3$ extractable-P (ppm)	25.8–1.0	7.8
DTPA extractable-Cu (ppm)	3.8–0.8	2.4
$CaCO_3$ equivalent (%)	4.5–0.1	1.3
HCO_3 (meq/l)	5.0–0.1	3.6
Ca + Mg (meq/l)	3.8–0.1	2.6
EC (mmhos/cm)	4.2–0.6	1.0

*pH, HCO_3, Ca + Mg and EC were determined from 1:2 soil water extract

MATERIALS AND METHODS

Soils Used and Methods of Cu Extraction. Twenty-one surface soils were collected from the rice growing areas of the Punjab, Pakistan just before crop sowing. They were air-dried, crushed in a wooden mortar to pass through a 2-mm sieve and analysed for physico-chemical characteristics (Table 1). The soils were clayey in texture, highly alkaline, moderately calcareous, rich in soluble salts, low in organic matter, and deficient in N and P. Available Cu in soils was determined by the following methods:

(a) *EDTA*. The extraction solution contained 0.02*M* EDTA. Five g soil was shaken with 25 ml solution of 30 min [12] on an end-over-end shaker and Cu in the filtrate was determined with an atomic absorption spectrophotometer [1].

(b) *DTPA*. The extraction solution contained 0.005*M* DTPA, 0.1*M* TEA (triethanolamine) and 0.01*M* $CaCl_2$ at pH 7.3. Ten g soil was shaken with 20 ml solution for 2 hr. Copper in the filtrate was determined with atomic abosrption spectrophotometer [11].

(c) *HCl*. Two g soil was mixed with 20 ml 0.1*M* HCl. After standing overnight, the mixture was shaken for 30 min. Copper in the filtrate was determined with atomic absorption spectrophotometer.

(d) *NH_4OAc*. Five g soil was shaken with 20 ml 1.0*M* NH_4OAc (pH 4.8) for 1 hr [7]. Copper in the extract was determined with atomic absorption spectrophotometer.

Studies on Response of Rice and Wheat to Cu Fertilization. Subsamples of various soils (5.5 kg for rice and 3.7 kg for wheat expts.) were filled in polythene-lined plastic pots of 20-cm dia. Basal fertilizer dressings consisted of N (as urea) at 75 and P (as KH_2PO_4) at 13 ppm. Copper sulphate was applied at 5 and 15 ppm to rice and wheat pots respectively. Each treatment with a control was replicated thrice. All the fertilizers were well mixed with the soils before sowing the plants. Four 15-day old nursery seedlings of rice (cv. Basmati-370) were transplanted in each pot in July 1973. The pots now submerged were kept flooded with deionized water throughout the growth period. For wheat experiments, 12 seeds of Chanab-70 wheat were sown in each pot in November 1973, and were thinned to 8 plants/pot a week later. The pots were maintained at field capacity by daily addition of deionized water throughout the period of plant growth.

The above ground parts of rice and wheat plants were harvested about 15 days before flower initiation (50 days rice and 60 days wheat). They were thoroughly washed in deionized water, dried, weighed and ground in a Wiley mill fitted with stainless-steel blades and other interior parts of the grinding chamber. One g portions of ground plant material were digested with 25 ml diacid mixture (redistilled HNO_3 and $HClO_4$ at 4:1) and Cu in the diluted digest was determined by atomic absorption spectroscopy [1]. Correlation coefficients (*r* values) between soil extractable Cu and plant Cu were calculated by using standard statistical procedures.

RESULTS AND DISCUSSION

Relationship Between Cu Uptake by Rice and Extractable Soil Cu. The amount of plant and soil Cu is shown in Table 2. The EDTA extractable Cu ranged from 1.4 to 7.0, DTPA from 0.8 to 3.8, NH_4OAc from 0.4 to 1.7 and HCl from 0.5 to 7.4 ppm. The efficiency of various methods for Cu extraction varied in the order of HCl > EDTA > DTPA > NH_4OAc. Almost similar order of their efficiency was found for extractable Zn [4]. Copper concentration in rice plants significantly correlated ($P < 0.01$) with total contents of Cu in plants (Table 3). None of the four methods of Cu extraction could, however, measure plant available Cu. The DTPA and NH_4OAc methods which successfully predict Cu for many upland crops on alkaline calcareous soils [8,20] showed little positive correlation either with concentration or total contents of Cu in flooded rice. Similarly HCl method could not correlate soil Cu with Cu concentration in plants. It, however, showed significant correlation ($P < 0.05$) with total Cu contents but the correlation coefficient was only 0.49. The EDTA Cu had also significant correlation ($P < 0.05$) with both the concentration and total Cu contents in rice plants but with *r* values of only 0.48 and 0.50 respectively. These values are so small that none of these two methods can be used with confidence for measuring plant-available Cu. Other workers drew similar conclusions from such low *r* values [2]. These methods could also not meet the criteria of distinguishing Cu deficient from nondeficient soils. When EDTA-extractable Cu was plotted against plant response ($P < 0.05$) to added Cu [19] on various soils (Fig. 1), several of the nondeficient soils scattered among the deficient ones and a critical Cu level, thus, could not be determined. Similarly HCl failed to distinguish between soils that responded to Cu application from those that did not (not shown).

To the best of our knowledge, studies on Cu extraction from soils in relation to its uptake by flooded rice have never been reported. Some preliminary studies on other micronutrients indicate that dilute acids, buffered salts and chelating compounds which successfully predict their availability for upland plant species failed to assess their

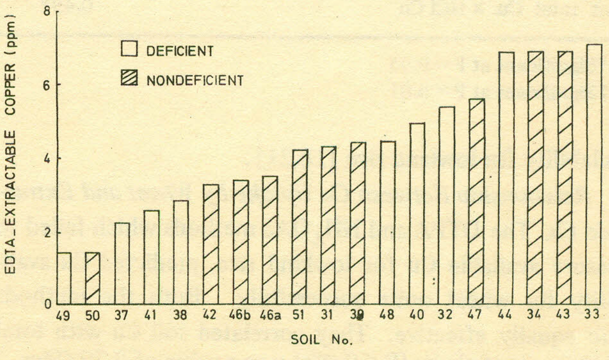

Fig. 1. Response of rice to soil applied $CuSO_4$ in relation to EDTA-extractable Cu.

Table 2. Relation of Cu uptake by rice with soil Cu extracted by four methods

Soil No.	Cu uptake by rice		Cu extracted by four methods			
	Cu concn. in plants (ppm)	Total Cu contents (μg/pot)	EDTA (ppm)	DTPA (ppm)	NH_4OAc (ppm)	HCl (ppm)
31	7.4	75.7	4.3	3.8	1.6	5.4
32	6.0	49.0	5.4	3.8	1.7	6.0
33	9.8	115.2	7.1	3.7	1.5	7.4
34	11.8	139.6	6.8	3.6	1.4	7.4
35	–	–	3.5	2.7	1.7	4.2
36	–	–	3.6	2.5	1.4	2.8
37	4.8	52.5	2.2	1.8	0.6	1.6
38	5.2	49.5	2.8	1.5	0.6	1.8
39	6.4	55.6	4.4	1.9	0.8	3.6
40	5.7	58.6	4.9	2.3	0.9	4.2
41	6.4	55.2	2.6	2.3	1.0	1.8
42	7.1	67.4	3.3	2.4	0.9	2.3
43	7.1	71.9	6.8	3.4	0.9	3.3
44	6.1	60.2	6.8	3.0	0.9	3.6
45	–	–	3.0	3.0	1.6	0.5
46 a	8.4	104.3	3.5	1.3	0.4	2.1
46 b	11.7	136.5	3.4	1.3	0.4	2.1
47	11.7	127.8	5.6	3.1	1.4	4.4
48	8.8	78.3	4.4	2.4	1.0	3.5
49	5.5	40.9	1.4	0.9	0.5	1.1
50	8.1	33.1	1.4	0.8	0.4	1.3
51	8.2	75.8	4.2	2.0	0.8	2.8

Table 3. Correlation between Cu uptake by rice and methods of Cu extraction.

Comparison	Correlation Coefficient
Plant Cu concn. × Plant total Cu	0.90†
Plant Cu concn. × EDTA Cu	0.48*
Plant Cu concn. × DTPA Cu	0.21
Plant Cu concn. × NH_4OAc Cu	0.22
Plant Cu concn. × HCl Cu	0.41
Plant total Cu. × EDTA Cu	0.50*
Plant total Cu. × DTPA Cu	0.32
Plant total Cu. × NH_4OAc Cu	0.27
Plant total Cu. × HCl Cu	0.49*

*Significant at P = 0.05
†Significant at P = 0.01

availability for lowland rice [18,21].

Relationship Between Cu Uptake by Wheat and Extractable Cu. The DTPA and NH_4OAc methods which failed to measure available Cu for lowland rice, predicted Cu availability for wheat quite successfully. Both the methods were equally effective. They correlated soil Cu with total Cu contents of plants ($P < 0.01$) by an *r* value of 0.70 (Figs. 2 and 3). The value increased to 0.84 when these methods

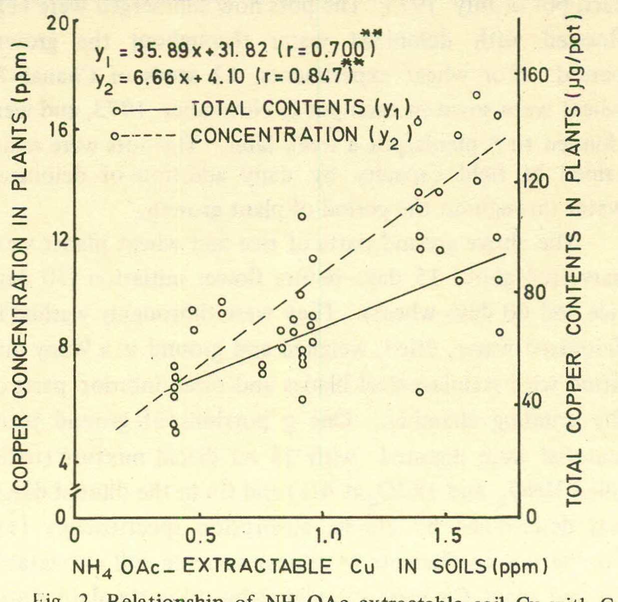

Fig. 2. Relationship of NH_4OAc-extractable soil Cu with Cu uptake by wheat plants. **Significant at $P < 0.01$

were correlated with Cu concentration in plants. These results are consistent with those of the other reports [19] where higher correlation coefficients were obtained when concentration rather than total contents of micronutrients in plants were considered. Since the present studies involv-

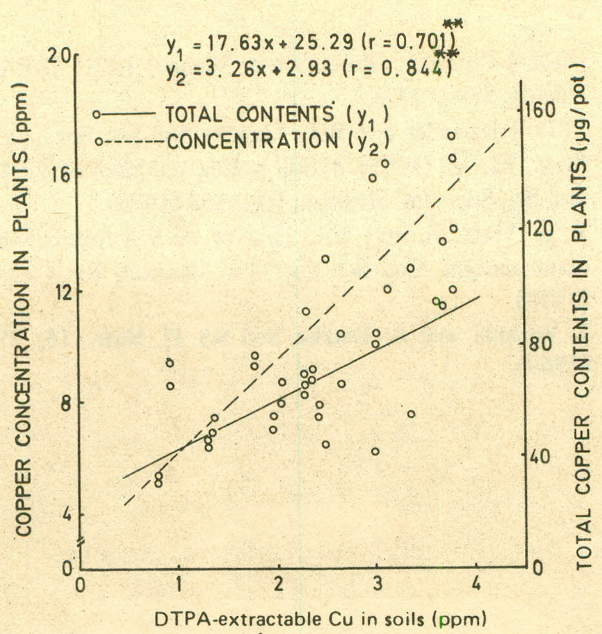

Fig. 3. Relationship of DTPA - extractable soil Cu with Cu uptake by wheat plants. **Significant at $P < 0.01$.

ed marginal to luxury Cu supplies (wheat responded to Cu application only on one soil), it may be necessary to evaluate these methods for Cu deficient soils. Other studies, however, indicate that these methods are effective on soils containing severe deficient to luxury Cu concentrations [7, 8, 11, 20] and they are being used as routine methods for detecting Cu deficiency for many upland crops on alkaline calcareous soils of the U.S.A. [20] and India [8]. Although both the methods are equally efficient in extracting plant-available Cu, DTPA method seems to have added advantage since both Cu and Zn [10] and possibly also Fe and Mn [20] can be determined in the same extract. This method, however, is only useful for laboratories equipped with aatomic absorption equipment. For other laboratories predigestion of DTPA is required before Cu colour is developed for colorimetric determination.

Critical Level of Soil-extractable Cu for Wheat. Since soils of the present studies were generally not Cu deficient and wheat responded to its application only on one soil, it was not possible to determine a precise critical level that separates responsive from nonresponsive soils. Further studies are needed especially by inclusion of a greater number of Cu responsive soils. However, the only soil (soil No. 50) where wheat responded to Cu application ($P < 0.05$) also exhibited the lowest concentrations of NH_4OAc and DTPA extractable Cu. If these levels, i.e. 0.8 and 0.4 ppm respectively, are considered general deficient levels, they are almost similar to that of NH_4OAc [8, 17] but slightly higher than DTPA critical Cu level reported earlier for upland crops on calcareous soils [20].

The present studies indicate that whereas a high correlation between soil and plant Cu existed for wheat, no such correlation occurred for lowland rice. The exact reasons of these discrepancies are not known. Flooded soil conditions of rice growth appear to be a predominant cause. Flooding induces severe chemical and electrochemical changes in calcareous soils. It does not decrease Cu solubility in the current soils [16]. It, however, enhances H, P, Fe, Mn, Ca, Mg and HCO_3 concentration in soil solutions [15, 16]. Most of these ions strongly depress Cu absorption by rice plants and probably account for its higher deficiency in rice than in wheat. Since concentrations of these ions in flooded soils generally exihibit little relationship with that in dry soils [9, 13], flooding can easily obscure the relationship between Cu uptake by rice and that extracted by various methods from dry soil samples. Correlation can perhaps be improved by inclusion of some of these ions in multiple regression equations.

Acknowledgements. This work was supported by IAEA under project No. 1407/RB and by USDA grant No. FG-Pa-221 (PK-ARS-3). The authors are grateful to Prof. J. F. Loneragan, University of Murdock, Western Australia, and Prof. A. Wallace, University of California, for critical reviewing the manuscript.

REFERENCES

1. A.E. Allan, Varian Aerograph, U.S.A. Print No. 12/69: A-10 (1969).
2. R.L. Blevins and H.F. Massey, Soil Sci. Soc. Am. Proc., **23**, 296 (1959).
3. F.M. Chaudhry, M. Sharif, A. Latif and R.H. Qureshi, Plant Soil, **38**, 573 (1973).
4. F.M. Chaudhry and M. Sharif, *in Nuclear Techniques in Research on Micronutrient Problems Pertaining to Crop Production,* (Proc. FAO/IAEA Panel and Research Coordination Meeting, Sept. 23–27, IAEA, Vienna, 1974) (in press).
5. Y. Dokiya, N. Owa and S. Mitsui, Soil Sci. Pl. Nutr., **14**, 169 (1968).
6. S.G. Dollar, D.R. Keeney and L.M. Walsh, J. Sci. Food Agr., **22**, 282 (1971).
7. J.G.A. Fiskell, *in* C.A. Black *et al.* (ed) Methods of Soil Analysis, Part 2, Agronomy, **9**, 1078 (1965).
8. J.S. Grewal, C. Lal, and N.S., Randhawa, Indian J. Agr. Sci., **39**, 877 (1969).
9. A. Islam and W. Islam, Plant Soil, **39**, 555 (1973).
10. M. A. Kausar, F. M. Chaudhry, S.M. Alam and M. Sharif Pakistan. J. Sci. Ind. Res., **21**, 39 (1978).
11. W.L. Lindsay and W.A. Norvell, Agron. Abstr., 84 (1969).
12. A. Oeien and G. Semb, Forsk. Fors. Landbrucket, **18**, 89 (1967).
13. M. O. Olomu, C.J. Racz and C.M. Cho, Soil Sci. Soc.

Am. Proc., **37**, 220 (1973).
14. Q.A. Osiname, F.E. Schulte and R.B. Corey. J. Sci. Food Agr., **24**, 1341 (1973).
15. F.N. Ponnamperuma. Advan. Agron., **24**, 29 (1972).
16. Rahmatullah, F.M. Chaudhry and A. Rashid, Plant Soil, **45**, 411 (1976).
17. W. L. Robertson, L. C. Thompson and F.G. Martin, Agron. J., **65**, 641 (1973).
18. J. W. B. Stewart, B. V. Fraize and F.M. Lapid, *in* Isotopes and Radiation in Soil-Plant Relationship including Forestry, (Proc. Symp. Vienna, 1971) IAEA, Vienna, 539 (1972).
19. J. F. Trierweiler and W.L. Lindsay, Soil Sci. Soc. Am. Proc., **33**, 49 (1969), *in* Soil testing and plant analysis, Soil Sci. Soc. Am. Madison, Wis., 153 (1973).
20. F. G. Viets, Jr. and W.L. Lindsay, *in* Soil testing and plant analysis, Soil Sci. Soc. Am. Madison, Wis., 153 (1973).
21. S. Yoshida and A. Tanaka, Soil Sci. Pl. Nutr., **15**, 75 (1969).