RESEARCH ARTICLE

Chemical speciation and suitablity of soil extractants for assessing Cu availability to maize (*Zea mays* L.) in acidic soils

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Abstract

Twenty surface (0-15 cm) samples of acidic soils were analysed for different chemical fractions of soil Cu namely; water soluble (WS), exchangeable (EX), lead displaceable (Pb-disp.), acid soluble (AS), Mn oxide occluded (MnOX), organically bound (OB), amorphous Fe oxide occluded (AFeOX), crystalline Fe oxide occluded (CFeOX), residual (RES) and also for soil extractable Cu using DTPA (pH 7.3), DTPA (pH 5.3), AB-DTPA (pH 7.6), Mehlich 3 (pH 2.0), Modified Olsen, 0.005 $MCaCl_2$, 1 M $MgCl_2$ extractants and ion exchange resins to understand the significance of different chemical fractions of Cu and suitability of soil extractant for predicting Cu availability to maize plants. Chemical fractions of Cu in acidic soils could be arranged in the following order: RES > MnOX > AFeOX > OB > CFeOX > AS > EX > Pb-disp. > WS. Chemical fractions of Cu in acidic soils and Cu extracted by the examined soil extractants were correlated with general soil properties. Water soluble (WS) and organically bound (OB) fractions of soil Cu showed significant and positive correlation with Cu uptake by maize. Among different examined soil extractants, DTPA (pH=5.3) and Mehlich 3 (pH = 2.0) showed significant and positive correlation with the dry matter yield, Cu concentration and uptake by maize plants in acidic soils.

Keywords: Chemical fractions, maize, soil extractants, copper uptake

1. Introduction

Copper is an essential micronutrient for the normal healthy growth and reproduction of higher plants and animals. The availability of Cu to higher plants is governed by a wide array of soil properties such as pH, redox potential, cation exchange capacity, texture, soil organic matter, Mn and Fe oxides content (Wu *et al.* 2010). In soils, Cu is associated with different inorganic and organic soil constituents. Chemical speciation is identification and determination of different chemical and physical forms of element present in a sample by sequential extraction technique (Grzebisz *et al*., 1997). Copper availability to plants is associated with its distribution among different soil chemical fractions or pools as these chemical fractions of soil Cu are likely to differ in their ease to release Cu in soil solution for plant absorption (Violante *et al*., 2010).

For the evaluation of Cu availability in soils, a variety of multi-nutrient soil extractants like 0.005 M diethylene triamine pentaacetic acid (DTPA, pH 7.3) (Lindsay and Norvell, 1978), 0.005 M DTPA (pH 5.3) (Norvell, 1984), ammonium bicarbonate-DTPA (AB-DTPA, pH 7.6) (Soltanpour and Workman, 1979), Mehlich 3 solution (pH 2.0) (Ostatek-Boczynski and Lee-Steere, 2012), $CaCl_2$, (Houba *et al*., 2000), 1M $MgCl_2$ (Tiller *et al*. 1972) are used. The suitability of a particular soil extractant for predicting the availability of Cu in soils can be decided on the basis of the relationship between the content of extractable soil Cu estimated by a given soil extractant and Cu concentration and uptake by plants. In the present investigation, we have attempted to relate the chemical speciation of soil Cu vis-a-vis extractable soil Cu using different multi-nutrient soil extractants to the concentration and uptake of Cu by maize grown in acidic soils to identify the Cu species playing important role in governing the availability of Cu to the plant and accordingly choose a multi-nutrient extractant for predicting Cu availability to plants.

2. Materials and Methods

Twenty surface (0-15 cm) soil samples were collected in bulk from the districts of Udham Singh Nagar, Nainital, Champawat of Uttarakhand Province and Moradabad district of Uttar Pradesh of India. The sampling area is located between 28o18'27.21" N to 29o24'55.07" N latitude and 79°27'10.75" E to 80°6'12.01" E longitude with the altitude ranging from 136.9 m to 1585.8 m above the mean sea level. The soils in the region belong to soil orders namely; alfisol, entisol, inceptisol and mollisol. Soil samples were air-dried under shade and crushed by wooden roller and passed through a 2-mm sieve. The soil samples were analyzed for mechanical analysis by Bouyoucous Hydrometer method, soil pH and electrical conductivity in 1: 2 soil water suspension and readily oxidisable C by modified Walkley and Black method as per standard procedures.

2.1. Chemical speciation of Copper in soils

Different chemical fractions of Cu in these soils were analysed following the sequential fractionation scheme proposed by Miller *et al*. (1986). As per this scheme, 2 g air dried soil sample was transferred in a polyethylene centrifuge tube in duplicate and sequentially extracted for different chemical fractions namely, water Soluble (WS) with 20 ml distilled water and 16 h shaking; exchangeable (EX) with 20 ml 0.5 M $Ca(NO_3)_2$ and 16 h shaking; Pb-displaceable (Pb-disp) with 20 ml 0.05 M $Pb(NO_3)_2$ + 0.1M $Ca(NO_3)_2$ and 16 h shaking; acid-soluble (AS) with 20 ml 0.44 M CH_3COOH + 0.1 M $Ca(NO_3)_2$ and 8 h shaking; Mn oxide occluded (MnOX) with 20 ml 0.01 M $NH_2OH.HCl$ + 0.1 M HNO_3 and 30 min. shaking; organically bound (OB) with 20 ml 0.1 M $K_4P_2O_7$ and 24h shaking; amorphous Fe-oxide occluded (AFeOX) with 20 ml 0.175 M $(NH_4)_2C_2O_4$

+ 0.1 M $H_2C_2O_4$ and 4 h shaking in darkness; crystalline Fe-oxide occluded (CFeOX) with 25 ml 0.1 M $H_2C_2O_4$ and 3h shaking under ultraviolet irradiation and residual (RES) by digesting the remainder material in HF+$HClO_4$ in Pt crucibles. During sequential extractions, the shaking was carried out on a temperature controlled orbital shaker at 120 rpm. After each extraction, the supernatant solution was separated by centrifuging the suspension in a centrifuge at 9000 rpm for 10 min.. The clear supernatant was decanted and stored in plastic vials in a refrigerator until analyzed for Cu by atomic absorption spectrophotometer (GBC Avanta M). The WS-Cu fraction was analyzed after pre-concentrating supernatant five times under acidic medium (0.1 M HCl).

2.2. Soil extractants

These soil samples were also extracted in duplicate by the following extractants :

(i)   DTPA solution (pH 7.3) - 0.005M DTPA + 0.1M TEA buffer + 0.01M $CaCl_2.2H_2$¬O, adjusted to pH 7.3; 10 g air-dried soil and 20 ml extractant were shaken for 2 h (Lindsay and Norvell, 1978).

(ii)   DTPA solution (pH 5.3) - 0.005M DTPA + 0.1M TEA buffer + 0.01M $CaCl_2.2H_2$¬O, adjusted to pH 5.3; 10 g air-dried soil and 20 ml extractant were shaken for 2 h (Norvell, 1984).

(iii)   AB-DTPA - 0.005 M DTPA +1 M Ammonium bicarbonate L^{-1} adjusted to pH 7.6 with NH_4OH; 5 g air-dried soil and 20 ml AB-DTPA were shaken for 15 min (Soltanpour and Workman, 1979).

(iv)   Mehlich 3 - 0.2 M $CH3COOH$ + 0.25 M NH_4F + 0.25 M NH_4NO_3+0.103 M HNO_3 + 0.001 M Ethylene diamine tetraacetic acid (EDTA) L^{-1} , adjusted to pH 2.0 with HCl; 5 g air-dried soil and 50 ml Mehlich 3 were shaken for 5 min (Ostatek-Boczynski and Lee-Steere, 2012).

(v)   Modified Olsen's - 0.5 M $NaHCO_3$ + 0.01 M EDTA + 0.1 g superfloc 127 L^{-1} adjusted to pH 8.5; 5 g air-dried soil and 25 ml of Modified Olsen's solution were shaken for 30 min (Hunter, 1975).

(vi)   0.005M $CaCl_2$ L^{-1}– 5 g air-dried soil and 25 ml 0.005M $CaCl_2$ were shaken for 1 h (Aitken *et al.*, 1987).

(vii)   1 M MgCl2 L^{-1}- 1.0 M $MgCl_2$ adjusted to pH 6.0 using $CH3COOH$; 5 g air-dried soil and 25 ml of 1 M $MgCl_2$ were shaken for 1 h (Tiller *et al.*, 1972).

For all extractions soil suspensions were shaken on a temperature controlled orbital shaker at 120 rpm and the contents were filtered.

(viii)   Ion-exchange resins - Twenty gram soil sample suspended in 40 ml water was treated with 2 milliequivalents. of both cation (Amberlite IRC 86) and anion exchange resin (Amberlite IRA 96) for 4h on a temperature controlled orbital shaker. The resins were separated and washed with distilled water and extracted in 30 ml of 2% HCl for 30 min.. The suspension was filtered.

The concentration Cu in the filtrates was determined using AAS.

2.3. Greenhouse experiment

A greenhouse experiment was conducted with maize (cv Pragati). Two and a half kg of each soil was filled in plastic pots on oven dry basis in duplicate. A basal dose of 30 mg N, 10.8 mg P and 20.8 mg K kg^{-1} soil was supplied to each pot through urea, potassium dihydrogen phosphate and potassium chloride in solution form. After thorough mixing of soil, the pots were refilled and irrigated with water and left for one week equilibration. When the soil moisture content in pots was near field capacity four healthy pre-soaked seeds of maize were sown in each pot. The pots were irrigated with tap water as and when required.

After 35d of emergence, the plants were harvested close to soil surface with a sharp Ni coated blade.

Harvested plants were sequentially washed in tap water, 0.1 N HCl and finally in distilled water and then dried in an electric oven at 60 °C for 48 h and dry matter yield was determined. Dried plants were finally ground and 1 g plant sample was ashed in a muffle furnace at 550 °C for 3 h. The ash was dissolved in 5 mL of 6 N HCl and diluted to 50 mL by distilled water. Plant samples were analyzed for Cu concentration by atomic absorption spectrophotometry (AvantaM, GBC). Copper uptake by maize plants (μg Cu pot^{-1}) was computed as product of dry weight of plants in gram per pot and Cu concentration in plant samples (μg Cu g^{-1}).

2.4. Statistical analysis

The data were subjected to simple correlation analysis and step-wise multiple regression analysis following the procedures outlined by Snedecor and Cochran (1967). The test of significance was conducted at 5 and 1% level of significance ($p \leq 0.05$). Path coefficient analysis was performed following the procedure outlined by Li (1956).

3. Results

These soils showed a wide variation in general properties. Soil texture varied from sandy clay loam to clay. Soil pH ranged from 4.57 to 6.83 while soil EC varied from 0.022 to 0.927 dS m^{-1}. Soil organic C content varied from 3.63 to 49.03 g kg^{-1} soil.

3.1. Chemical fractions of Cu

The ranges, means and standard deviations for the different chemical fractions of Cu in these acidic soils are presented in Table 1. Among different chemical fractions of Cu, the water soluble Cu fraction (WS) showed relatively the lowest values; the observed range was 0.004 to 0.033 mg kg^{-1} soil. In theses soils the WS- fraction tended to be generally higher in soils having higher clay content and higher acidity. The contents of EX- and Pb disp.- fractions varied from <0.001 to 1.95 and <0.001 to 0.62 mg kg^{-1} soil, respectively indicating a lower tendency of Cu^{2+} to exist as exchangeable cation on soil exchange complex. Though these soils had no free carbonates yet the content of AS-fraction ranged from 0.10 to 2.02 mg kg^{-1} soiland this could be related to the presence of Cu-fulvates in the extract which were dissolved due to the acidic pH of the extracting solution used at this step. The OB-fraction ranged from 0.55 to 4.02 mg kg^{-1} soil and the higher values of this fraction were recorded for soils having relatively higher organic C content. Among oxide bound fractions, the content of MnOX-, Am. FeOX- and CFeOX- fractions varied from 0.24 to 3.46, <0.001 to 4.52 and 0.20 to 2.48 mg kg-1 soil, respectively. These observations indicated a higher preference of Mn oxides to hold Cu^{2+} as compared to Fe oxides. The residual fraction (RES) exhibited the highest values which ranged between 13.00 to 187.38 mg kg^{-1} soil. Based on the mean values the different chemical fractions of Cu in these soils could be arranged in the following order: RES > MnOX > AFeOX > OB > CFeOX > AS > EX > Pb-disp. > WS. Simple correlation analysis was carried out between different chemical fractions of Cu and soil properties to understand the influence of general soil properties on the distribution of soil Cu into different chemical fractions of Cu (Table 2). The results revealed that water soluble fraction showed a significant and positive correlation with clay content; the simple correlation coefficient value (r) being 0.478; significant at $p \leq 0.05$. Organically bound Cu fraction showed a significant and positive correlation with organic C; the r was 0.463; significant at $p \leq 0.05$. However, the rest chemical fractions of Cu failed to show statistically significant correlation with any soil properties.

Table 1. The range, mean and standard deviation values of different chemical fractions of Cu in acidic soils (n = 20)

hemical fractions of Cu (mg kg^{-1} soil)	Range (mg kg^{-1} soil)		Mean (mg kg^{-1} soil)	S.D.
	Min.	Max.		
Water Soluble Cu (WS-Cu)	0.004	0.033	0.013	0.008
Exchangeable Cu (EX. Cu)	<0.001	1.950	0.604	0.565
Lead – displaceable Cu (Pb disp.- Cu)	<0.001	0.620	0.082	0.219
Acid Soluble Cu (AS-Cu)	0.100	2.020	0.691	0.502
Mn-oxide occluded Cu (Mn OX-Cu)	0.240	3.460	1.934	1.024
Organically bound Cu (OB-Cu)	0.550	4.020	1.802	0.833
Amorphous Fe-oxide occluded Cu (Am. FeOX-Cu)	<0.001	4.520	1.878	0.919
Crystalline Fe-oxide occluded Cu	0.200	2.480	1.004	0.617
Residual Cu (RES.-Cu)	13.000	187.380	44.232	38.944

Table 2. Simple correlation coefficients (r) between different chemical fractions of Cu and general soil properties

Soil Cu fractions	SOIL PROPERTIES					
	Sand (%)	Silt (%)	Clay (%)	pH	EC	Organic C (g kg^{-1})
WS-Cu	-0.265	-0.239	0.455*	-0.052	-0.224	-0.131
EX-Cu	-0.193	0.178	0.069	-0.098	0.205	-0.096
Pb-disp.-Cu	0.262	-0.001	-0.274	0.127	0.027	-0.073
AS-Cu	0.098	0.137	-0.205	-0.186	0.128	-0.275
Mn OX-Cu	0.01	0.368	-0.285	-0.072	0.006	0.233
OB-Cu	0.113	0.236	-0.295	-0.128	0.035	0.463*
AFeOX-Cu	0.282	0.079	-0.354	-0.15	-0.024	0.416
CFeOX-Cu	0.066	0.025	-0.087	-0.056	0.475	-0.026
RES-Cu	-0.123	-0.349	0.389	-0.227	-0.158	-0.31

** Significant at $p \leq 0.01$, * Significant at $p \leq 0.05$

3.2. Soil extractable Cu estimated by different extractant

The ranges, means and standard deviations for soil extractable Cu estimated by the different extractants are presented in Table 3. These soil extractants could be arranged in the following order of their Cu extraction power: Mehlich 3 > AB – DTPA (pH 7.6) > DTPA (pH 7.3) > DTPA (pH 5.3) > Modified Olsen's > 1 M MgCl$_2$ > 0.005 M CaCl$_2$ > Resin extractable.

As compared to simple salt solutions like 0.005 M CaCl$_2$, 1 M MgCl$_2$, or milder ion exchange resins, the extractants containing chelating agents like Mehlich 3, AB – DTPA (pH 7.6), DTPA (pH 7.3, DTPA (pH 5.3) and Modified Olsen's extractants extracted higher Cu concentrations from these soils. Simple correlation coefficients (r) between soil extractable Cu estimated by different extractants and soil properties are presented in Table 4.

Table 3. The range, mean and standard deviation of soil extractable Cu (mg kg^{-1} soil) estimated by different soil extractants (n = 20)

Soil extractable Cu (mg kg^{-1} soil)	Range (mg kg^{-1} soil)		Mean (mg kg^{-1} soil)	S.D.
	Min.	Max.		
DTPA (pH = 7.3)	0.11	3.77	1.42	0.97
DTPA (pH = 5.3)	0.12	3.48	1.27	0.94
AB-DTPA (pH = 7.6)	0.45	7.94	3.19	2.03
Mehlich 3 (pH =2.0)	1.63	22.27	5.90	4.66
Modified Olsen's	0.18	3.39	1.05	0.80
0.005 M CaCl2	0.02	0.16	0.07	0.04
1 M MgCl2	0.01	0.79	0.15	0.16
Ion Exchange Resins	0.02	0.09	0.05	0.02

Table 4. Simple correlation coefficients (r) between general soil properties and extractable Cu estimated by different soil extractants

General soil properties	Soil Extractable Cu (mg kg^{-1})							
	DTPA (pH = 7.3)	DTPA (pH 5.3)	Amm. Bicarbonate -DTPA (pH = 7.6)	Mehlich3 (pH = 2.0)	Mod. Olsen's	0.005 M CaCl$_2$	1 M MgCl$_2$	Ion Exchange Resins
Sand	0.008	-0.003	0.000	0.157	-0.092	0.050	0.508*	-0.277
Silt	0.439	0.142	0.383	0.147	0.399	0.199	0.039	0.353
Clay	-0.336	-0.102	-0.287	-0.274	-0.202	-0.201	-0.560*	0.026
pH	-0.121	0.114	-0.046	0.075	-0.236	-0.030	-0.289	-0.235
EC	0.201	0.130	0.157	0.043	0.164	0.048	-0.149	-0.166
OC	0.233	-0.051	0.148	0.103	-0.106	0.100	-0.156	0.310

** Significant at $p \leq 0.01$, * Significant at $p \leq 0.05$.

Table 4. Simple correlation coefficients (r) between general soil properties and extractable Cu estimated by different soil extractants

Chemical fractions of Soil Cu	Soil Extractants							
	DTPA (pH = 7.3)	DTPA (pH= 5.3)	AB-DTPA (pH= 7.6)	Mehlich3 (pH = 2.0)	Mod. Olsen's	0.005 M CaCl$_2$	1M MgCl$_2$	Ion exchange resins
WS-Cu	-0.054	0.365	-0.011	0.069	-0.078	-0.003	-0.156	-0.110
EX-Cu	-0.055	-0.112	-0.202	-0.123	-0.007	-0.195	0.052	-0.240
Pb-disp.-Cu	0.647**	0.793**	0.753**	0.573**	0.703**	-0.002	0.061	0.365
AS-Cu	0.467*	0.527*	0.461*	0.274	0.639**	-0.172	0.278	0.309
Mn OX-Cu	0.341	0.505*	0.360	0.479*	0.156	0.074	0.179	0.394
OB-Cu	0.324	0.263	0.294	0.581**	0.025	0.340	0.280	0.319
AFeOX-Cu	0.342	0.408	0.375	0.599**	0.147	0.078	0.350	0.509*
CFeOX-Cu	0.552*	0.489*	0.580**	0.638**	0.508*	0.150	0.207	0.435
RES-Cu	-0.400	-0.347	-0.330	-0.005	-0.263	0.136	0.289	0.026

Among soil properties, 1 M MgCl$_2$ extractable Cu showed a significant and negative correlation with clay content but a positive correlation with sand content; both 'r' values were significant at $p \leq 0.05$. The quantity of Cu extracted by other soil extractants failed to show any statistically significant relationships with measured soil properties.

In order to understand the bearing of different chemical fractions of Cu on soil extractable Cu estimated by different soil extractants, simple correlation analysis was also performed between soil extractable Cu and different chemical fractions of Cu in acidic soils and the values of simple correlation coefficients (r) are presented in Table 5. Among different soil extractants, Mehlich-3 (pH = 2.0) showed a significant and positive correlation with Pb-displaceable Cu (r = 0.573; significant at $p \leq 0.01$), Mn oxide occluded Cu (r = 0.479; significant at $p \leq 0.05$), organically bound Cu (r = 0.581; significant at $p \leq 0.01$), amorphous Fe oxide occluded Cu (r = 0.599; significant at $p \leq 0.01$) and crystalline Fe oxide occluded Cu fraction (r = 0.638; significant at $p \leq 0.01$). The DTPA (pH = 7.3), DTPA (pH = 5.3) and AB-DTPA (pH = 7.6) extractable Cu showed a significant and positive correlation with Pb-displaceable Cu-; acid soluble Cu and crystalline Fe oxide occluded Cu fractions. The DTPA (pH = 5.3) extractable Cu also showed a significant and positive correlation with Mn oxide occluded –Cu. The modified Olsen extractable soil Cu had a significant and positive correlation with Pb-displaceable Cu and acid soluble Cu fractions. Ion exchange resin extractable Cu showed a significant and positive correlation with amorphous Fe oxide occluded Cu (r = 0.509; significant at $p \leq 0.05$).

3.3. Relationships between chemical fractions of soil Cu and dry matter yield; Cu concentration and uptake by maize

The dry matter yields of maize (cv. Pragati) raised on these soils at 35 d after emergence varied from 0.2 to 7.7 g pot^{-1} (mean value = 2.2 g pot^{-1}), Cu concentration in plants ranged from 3.70 to 10.10 µg g^{-1} (mean = 5.99 µg g^{-1}) and Cu uptake ranged from 0.70 to 77.50 µg pot^{-1} (mean = 15.43 µg pot^{-1}).

Table 5. Simple correlation coefficient (r) between chemical fractions of Cu and Cu extracted by different extractants in acidic soils

Chemical fractions of Soil Cu	Soil Extractants							
	DTPA (pH = 7.3)	DTPA (pH= 5.3)	AB-DTPA (pH= 7.6)	Mehlich3 (pH = 2.0)	Mod. Olsen's	0.005 M CaCl₂	1M MgCl₂	Ion exchange resins
WS-Cu	-0.054	0.365	-0.011	0.069	-0.078	-0.003	-0.156	-0.110
EX-Cu	-0.055	-0.112	-0.202	-0.123	-0.007	-0.195	0.052	-0.240
Pb-disp.-Cu	0.647**	0.793**	0.753**	0.573**	0.703**	-0.002	0.061	0.365
AS-Cu	0.467*	0.527*	0.461*	0.274	0.639**	-0.172	0.278	0.309
Mn OX-Cu	0.341	0.505*	0.360	0.479*	0.156	0.074	0.179	0.394
OB-Cu	0.324	0.263	0.294	0.581**	0.025	0.340	0.280	0.319
AFeOX-Cu	0.342	0.408	0.375	0.599**	0.147	0.078	0.350	0.509*
CFeOX-Cu	0.552*	0.489*	0.580**	0.638**	0.508*	0.150	0.207	0.435
RES-Cu	-0.400	-0.347	-0.330	-0.005	-0.263	0.136	0.289	0.026

** Significant at $p \leq 0.01$, * Significant at $p \leq 0.05$

Table 6. Simple correlation coefficients between different chemical fractions of soil and dry matter yield (g pot⁻¹), plant Cu content (µg g⁻¹) and Cu uptake (µg pot⁻¹)

Chemical fractions of soil Cu	Dry Matter (g pot⁻¹)	Cu content (µg g⁻¹)	Cu Uptake (µg pot⁻¹)
WS-Cu	0.498*	0.477*	0.556*
EX-Cu	0.233	-0.257	0.222
Pb-disp.-Cu	0.126	0.722**	0.249
AS-Cu	0.150	0.603**	0.204
Mn OX-Cu	0.131	0.548*	0.264
OB-Cu	0.377	0.412	0.467*
AFeOX-Cu	0.218	0.364	0.338
CFeOX-Cu	0.285	0.525*	0.378
RES-Cu	-0.074	-0.046	-0.037

** Significant at $p \leq 0.01$, * Significant at $p \leq 0.05$

The simple correlation coefficients between different chemical fractions of Cu in acidic soils and dry matter yield, Cu concentration and uptake by maize are depicted in Table 6. Dry matter yield of maize showed a significant and positive correlation with water soluble fraction of soil Cu at $p \leq 0.05$. Copper concentration in maize plants showed significant and positive correlation with Pb-displaceable and acid soluble fractions of soil Cu at $p \leq 0.01$ and also with water soluble-; Mn oxide bound- and crystalline Fe oxide bound- fractions of soil Cu at $p \leq 0.05$. Copper uptake by maize plants showed a significant and positive correlation with water soluble- and organically bound-fractions of soil at $p \leq 0.05$ with Cu uptake.

3.4. Relationships between Dry matter yield; Cu concentration and uptake by maize and extractable soil Cu estimated by different soil extractants

Simple correlation coefficients (r) computed between extractable content of soil Cu estimateded by different soil extractants and dry matter yield, Cu concentration and uptake of maize plants are depicted in Table 7. The DTPA (pH = 5.3) extractable soil Cu (r = 0.450) and Mehlich-3 extractable Cu (r = 0.463) showed a significant and positive correlation with dry matter yield of maize and both 'r' values were significant at

$p \leq 0.05$. The DTPA (pH 7.3)-, DTPA (pH 5.3)-, AB-DTPA (pH = 7.6) and Mehlich 3 extractable soil Cu showed a significant and positive correlation with Cu concentration in maize plants; all 'r' values were significant at $p \leq 0.01$. Modified Olsen extractable Cu also showed a significant and positive correlation with Cu concentration in maize (r = 0.522; significant at $p \leq 0.05$). The DTPA (pH 5.3) extractable Cu and Mehlich 3 extractable Cu showed a significant and positive correlation Cu uptake and 'r' values were significant at $p \leq 0.05$ and ≤ 0.01, respectively.

Table 7. Correlation coefficients for Cu extracted by different soil test methods, dry matter yield, plant Cu content ($\mu g\ g^{-1}$) and Cu uptake ($\mu g\ pot^{-1}$)

Soil Extractants	Dry Matter (g pot^{-1})	Cu content ($\mu g\ g^{-1}$)	Cu Uptake ($\mu g\ pot^{-1}$)
DTPA (pH = 7.3)	0.257	0.623**	0.329
DTPA (pH = 5.3)	0.450*	0.838**	0.544*
AB-DTPA (pH = 7.6)	0.221	0.661**	0.310
Mehlich 3 (pH= 2.0)	0.463*	0.593**	0.581**
Mod. Olsen's	0.142	0.522*	0.176
0.005 M CaCl$_2$	0.091	0.078	0.098
1 M MgCl$_2$	0.044	-0.068	0.012
Ion Exch. Resin ext.	0.009	0.140	0.064

** Significant at $p \leq 0.01$, * Significant at $p \leq 0.05$.

4. Discussion

The distribution of heavy metals among different chemical fractions is jointly governed by the nature of metal and the array of soil properties. The low magnitude of WS-, Pb-disp.- and EX- Cu fractions in these soils indicated a very sorption capacity of acid soils for Cu owing to higher electronegativity of Cu^{+2} ions as compared to Zn^{+2} and Mn^{2+} ions which resulted in a stronger covalent bond with O^{2-} present in minerals. This fact was also supported by the magnitude of concentrations of

Cu associated with RES-, MnOX- and AFeOX- fractions as observed earlier by other researchers as well (McLaren and Crawford, 1973; Ma and Rao, 1997; Fathi *et al.*, 2014). A much higher magnitude of OB-fraction as compared to EX-fraction revealed that in contrast to clays the soil organic matter offers specific sites to form inner sphere complexes with humic substances (McBride, 1994). A significant and positive correlation between water soluble Cu fraction with clay content and between OB-fraction of Cu with organic C was supportive of such phenomenon.

Among the multi-nutrient soil extractants used in the study, Mehlich 3 solution demonstrated the highest capacity of Cu extraction in comparison to the other extractants which could be attributed to low pH of extractant (Abreu *et al*., 1996; Ostatek-Boczynski and Lee-Steere, 2012) and the presence of EDTA; a chelating agent which lowered activity of Cu ions to effect dissolution of labile forms of Cu from soil sample (Vidal-Vázquez *et al*., 2005). A significant and negative correlation between 1 M $MgCl_2$ extractable Cu with clay content but a positive correlation with sand content indicated low content of specifically bound Cu in soils of high clay content which incidentally also had lower content of organic C (r = -0.328, significant at $p \leq 0.075$).

Different soil extractants used in the study also varied in their power to effect release of Cu drom different chemical fractions and accordingly showed their dependence of the respecctive chemical fractions of Cu in acidic soils. The Mehlich 3 extractant could release Cu from Pb-dis.-, MnOX-, OB-, AFeOX- and CFeOX-fractions. Other extractants like DTPA (pH = 7.3), DTPA (pH = 5.3) and AB-DTPA (pH = 7.6) extracted Cu only from Pb-dis.-, AS- and CFeOX-fractions while DTPA (pH = 5.3) also extracted Cu from MnOX– fraction; an important sink of Cu in soils (Fathi *et al*., 2014) due to relatively lower pH as compared to DTPA (pH = 7.3) and AB-DTPA (pH = 7.6). Thus, soil extractants containing chelating agents could release Cu from inorganic surfaces retaining Cu by inner sphere complexation. However, Mehlich 3 extractant which had EDTA as chelating agent and also the lowest pH (2.0) could release some fraction of organically bound Cu from soils. On the other hand, the modified Olsen's extractant could extract Cu mainly from Pb-dis.- and AS- fractions while the ion exchange resin extracted Cu from only AFeOX- fraction due to non-acidic nature. The 0.005 M $CaCl_2$ extractable Cu; which extracted the lowest

mean content of soil Cu, did not to show any statistically significant relationship with any chemical fraction of soil Cu. Micronutrient availability to plants can be measured in the direct uptake experiments, or estimated with techniques that correlate quantities of micronutrients extracted chemically from soils to plant uptake and response to micronutrient fertilization (White and Zasoski, 1999). The observed significant and positive correlation between dry matter yield, Cu concentration and uptake of maize with water soluble fraction of soil Cu at $p \leq 0.05$ indicated the significance of soil solution concentration of Cu from crop production point of view. Further, a significant correlation between Cu concentration in maize with Pb-displaceable-, acid soluble-, Mn oxide bound- and crystalline Fe oxide bound- fractions of soil Cu showed that these Cu fractions could be of significance in acid soils. A significant and positive correlation of Cu uptake by maize with the water soluble- and organically bound-fractions of soil signified that water soluble- and organically bound- fractions of soil Cu constituted labile pool of Cu and control the bioavailability of Cu to growing plants (Gunken *et al*., 2003). Rupa and Shukla (1999) also recorded statistically significant and positive correlations in the case of water soluble, exchangeable and organic matter bound fractions of Cu with the dry matter yield, concentration and uptake of Cu by rice. The significance of Fe and Mn oxide occluded Cu fractions from bioavailability point of view was relatively less (Raghupathi and Vasuki, 1992).

Among the soil extractants, DTPA (pH = 5.3) and Mehlich-3 extractable Cu showed a significant and positive correlation with dry matter yield of maize. The DTPA (pH 7.3)-, DTPA (pH 5.3)-, AB-DTPA (pH = 7.6), Mehlich – 3 and modified Olsen - extractable soil Cu showed a significant and positive correlation with Cu concentration in maize plants. The DTPA (pH 5.3) extractable Cu and Mehlich-3 extractable

Cu showed a significant and positive correlation Cu uptake by maize plants. This revealed the suitability of DTPA (pH 5.3) and Mehlich 3 extractants for assessing the bioavailability of Cu in acid soils. Several other workers (Borkert *et al*., 2004; Ostatek-Boczynski and Lee-Steere, 2012; Kantek and Korzeniowska, 2013; Gediga *et al*., 2014) also recorded the suitability of Mehlich 3 to assess plant available Cu.

5. Conclusions

In acid soils, residual Cu was the most dominant fraction followed by Mn oxide occluded-; amorphous Fe oxide bound-; organically bound-; crystalline Fe oxide bound-; acid soluble-; exchangeable-; Pb displaceable- and water soluble-Cu fractions in that order. From plant availability point of view, water soluble- and organically bound- Cu fractions were the most important in the acidic soils. Among the tested soil extractants, DTPA (pH 5.3) and Mehlich 3 (pH 2.0) appeared to be the most promising soil extractants to assess the availability of Cu in acid soils.

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