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Impact of Manure and Fertilizers on Chemical Fractions of Zn and Cu in Soil under Rice-Wheat Cropping System

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The present study was conducted in a long-term field experiment (in progress since *kharif* 2009-10) at Department of Soil Science, Punjab Agricultural University, Ludhiana. The objective of the study was to investigate the impact of organic manure (biogas slurry) and fertilizer application on chemical fractions of zinc (Zn) and copper (Cu) under rice-wheat system. Treatments applied to rice-wheat system were arranged in a split-plot design with three replications. Main plot treatments applied to rice were four combinations of manure and fertilizers *viz*., nitrogen (N) @ 120 kg ha^{-1} without manure and phosphorus (P) fertilizer ($M_0N_{120}P_0$), N @ 80 kg ha^{-1}, P @ 30 kg ha^{-1} and manure @ 6 t ha^{-1} ($M_6N_{80}P_{30}$), N @ 120 kg ha^{-1}, P @ 30 kg ha^{-1} and no manure ($M_0N_{120}P_{30}$) and N @ 80 kg ha^{-1}, manure @ 6 t ha^{-1} without P-fertilizer ($M_6N_{80}P_0$). Three sub-plot treatments applied in wheat included N @ 120 kg ha^{-1}, K @ 30 kg ha^{-1} and three levels of P-fertilizer *viz*., P @ 30 kg ha^{-1} (P_{30}), P @ 60 kg ha^{-1} (P_{60}) and P @ 0 kg ha^{-1} (P_0). Results revealed that the concentration of Zn and Cu was higher under the treatments where biogas slurry was applied along with fertilizers as compared to the sole application of fertilizers. The increase in water soluble and exchangeable (WSEX) fractions in organically treated plots indicated their mobilization from occluded fractions *viz*., crystalline iron oxide (CFeOX) and amorphous iron oxide (AFeOX) bound fractions. The residual (RES) fraction was the dominant portion of total Zn and Cu content of soil; whereas the contribution of WSEX fraction was limited. Organic matter bound fractions of Zn and Cu were the most important contributing towards their uptake by both rice and wheat crops.

Key words: Chemical fractions, zinc, copper, biogas slurry manure, NPK, rice-wheat system

Production of food grains is increasing year after year due to intensive cultivation, thereby attenuating a huge amount of macronutrients along with micronutrients. In several areas with intensive cropping, zinc (Zn) deficiency has been observed along with the deficiencies of other micronutrients. The Zn and iron (Fe) concentrations are activated differentially and the dynamics of their inter-conversion from one fraction to the other is hastened due to decomposition of added manures (Dhaliwal *et al*. 2013). The organic matter (OM) bound fractions of Zn and copper (Cu) increased with application of farmyard manure (FYM) in rice-wheat system. However, application of FYM resulted in increase and redistribution of Zn and Cu from occluded forms (carbonates and crystalline iron oxides) to readily available (water-soluble plus exchangeable fraction) and potentially available (organic fraction, manganese oxides and amorphous iron oxides) forms in soil (Sekhon *et al*. 2006). Dhaliwal (2008) observed that green manure (GM) and soil applied manganese (Mn) to rice-wheat system increased the DTPA-extractable, water soluble plus exchangeable and Mn specifically adsorbed fractions on the inorganic sites whereas, Mn held on organic sites and oxide bound surfaces decreased.

The effect of OM and phosphorus (P) on distribution of Cu in Alfisols under submergence was studied and it was found that Cu was mobilized from water soluble and exchangeable (WSEX), organically complexes and crystalline iron oxide (CFeOX) fractions to amorphous iron oxide (AFeOX) and residual (RES) fractions. The rate of mobilization was maximal from CFeOX to RES fraction during initial 15 days period. Application of OM decelerated Cu

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transformation and from RES fraction and increased its content in AFeOX fraction. It also retarded WSEX Cu markedly. The Cu dynamics was not significantly regulated by P application (Saha *et al*. 2000). Results on transformation of fractions of Zn in selected treatments in a long-term experiment under maize-wheat cropping system showed that among the various fractions of Zn, RES-Zn was the dominant fraction of total-Zn in the maize and wheat plots (Bahera *et al*. 2008). It was also reported that compared to natural site, cultivation seemed to have caused a switch from AFeOX-Cu to CFeOX-Cu. The retarding AFeOX-Cu and increasing CFeOX-Cu in the cultivated field were likely due to increasing crystallization of Fe oxide induced by cultivation. Because cultivation causes the loss of OM which inhibits Fe crystallization, this can facilitate the transformation of AFeOX-Cu to CFeOX-Cu because CFeOX-Cu is essentially Cu occluded in crystalline Fe oxide (Agbenin and Henningsen 2004). The available Zn and Cu levels in cropped treatments were lower as compared to the fallow treatment, presumably due to the depletion of these micronutrients from the system through crop uptake and harvest (Wei *et al*. 2006). The availability of WSEX-Zn and Cu decreased with time as this form is exhausted from the soil solution (Ojha *et al*. 2018).

The knowledge of various fractions of nutrients present in soil and conditions under which these become available to plants is pre-requisite in assessing their availability to plants. The information regarding the transformation of micronutrient cations especially Zn and Cu among different chemical fractions with the combined application of organic manure (biogas slurry) and fertilizers is lacking and needs to be investigated. Therefore, the present study was conducted with the aim to study the impact of manure and fertilizers on chemical fractions of Zn and Cu under rice-wheat system. It is expected to reveal the chemical behavior of Zn and Cu under different fractions in the soil which is the basis for their availability to plants.

Materials and Methods

Study site, climate and soil characteristics

The study was conducted on an on-going field experiment to investigate the impact of manure and fertilizers on chemical fractions of Zn and Cu under rice-wheat cropping system (in progress since *kharif* 2009-10). The experimental site was located at the research farm of Punjab Agricultural University, Ludhiana, India at an elevation of 247 m above mean sea level and lie at 30°54′ latitude and 75°40′ longitude, which represents the central agro-climatic zone of Punjab. The climate is sub-tropical to tropical with a long dry season from late September to early June and wet season from July to early September. The soil of experimental field was sandy loam in texture having pH 6.71, electrical conductivity (EC) 0.17 dS m^{-1}, organic carbon (OC) 3.3 g kg^{-1}, and available nitrogen (N), phosphorus (P) and potassium (K) of 275, 23 and 184 kg ha^{-1}, respectively. Each treatment was replicated thrice in a plot size of 11×6 m^2. The experiment was laid out in a split-plot design with four main plot and three sub-plot treatments. Main plot treatments applied to rice were four combinations of manure and fertilizers *viz*., N @ 120 kg ha^{-1} without manure and P fertilizer ($M_0N_{120}P_0$), N @ 80 kg ha^{-1}, P @ 30 kg ha^{-1} and manure @ 6 t ha^{-1} ($M_6N_{80}P_{30}$), N @ 120 kg ha^{-1}, P @ 30 kg ha^{-1} and no manure ($M_0N_{120}P_{30}$) and N @ 80 kg ha^{-1}, manure @ 6 t ha^{-1} without P-fertilizer ($M_6N_{80}P_0$). The three sub-plot treatments in wheat included three levels of P-fertilizer *viz*., P @ 30 kg ha^{-1} (P_{30}), P @ 60 kg ha^{-1} (P_{60}) and P @ 0 kg ha^{-1} (P_0) along with N @ 120 kg ha^{-1} and K @ 30 kg ha^{-1} (Table 1).

Laboratory analysis

The particle size distribution of soil was determined using International pipette method (Day 1965) and soil pH and EC in 1:2 soil-water suspension with the help of glass electrode pH meter (Jackson 1973). Organic carbon was determined by rapid titration method given by Walkley and Black (1934). Available N was determined by alkaline permanganate

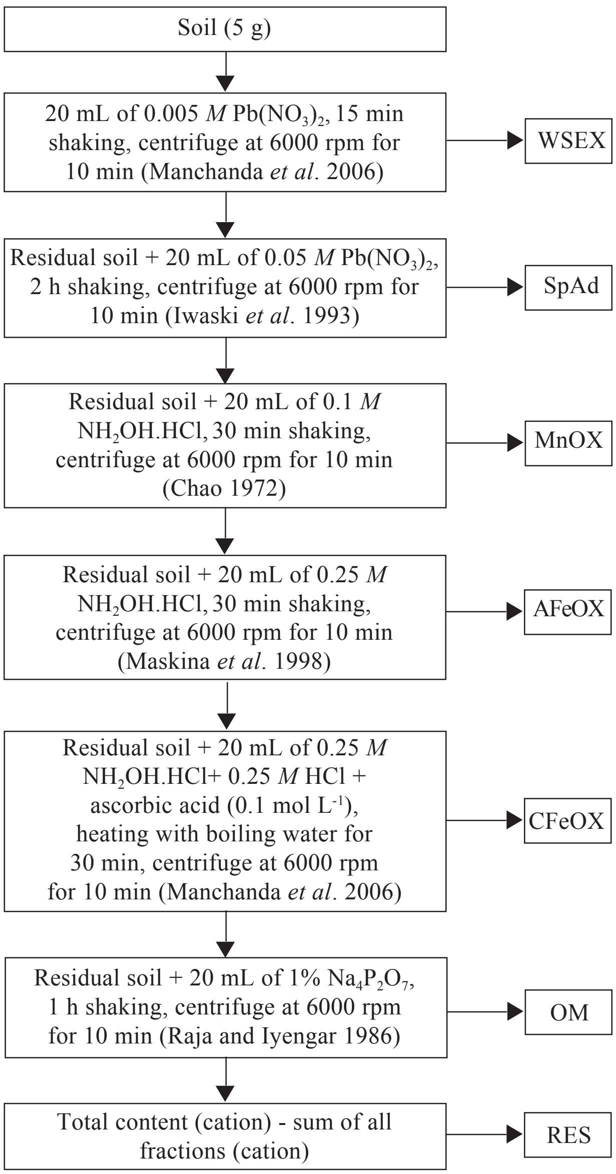
Table 1. Treatment details of long-term experiment on rice-wheat system

Treatments	Rice		Wheat	
	Manure (t ha^{-1})	N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	P_2O_5 (kg ha^{-1})
T_1	0	120	0	0
T_2	0	120	0	30
T_3	0	120	0	60
T_4	6	80	30	0
T_5	6	80	30	30
T_6	6	80	30	60
T_7	0	120	30	0
T_8	0	120	30	30
T_9	0	120	30	60
T_{10}	6	80	0	0
T_{11}	6	80	0	30
T_{12}	6	80	0	60

method (Subbiah and Asija 1956), available P by Olsen *et al.* (1954), available K by extraction with 1 N ammonium acetate at pH 7 (Jackson 1973).

Fractionation scheme of Zn and Cu

The processed surface (0-15 cm) soil samples were used to fractionate the Zn and Cu into following chemical forms as per sequential extraction procedure described below and the Zn and Cu in the extract was analyzed by atomic absorption spectrophotometer.



Statistical analysis

The experiment was laid out in a split-plot design with three replications. Least significant difference was used to compare the treatments effect at *P<0.05* (Panse and Sukhatme 1985).

Results and Discussion

Effect of manure and fertilizers on chemical fractions of Zn

Various chemical fractions of Zn in surface (0-15 cm) soil samples collected after harvesting of rice are presented in table 2. The WSEX-Zn content ranged from 1.30 to 1.50 mg kg^{-1} under different treatment combinations. The content of WSEX-Zn was significantly superior over other treatments under the integrated use of manure (biogas slurry) and inorganic fertilizers to the rice crop *i.e.* $M_6N_{80}P_{30}$ and $M_6N_{80}P_0$. Both $M_6N_{80}P_{30}$ and $M_6N_{80}P_0$. However, WSEX-Zn content was lower under the treatments where fertilizers were applied alone without manure *i.e.* $M_0N_{120}P_0$ and $M_0N_{120}P_{30}$. Tiecher *et al.* (2013) and Boguta and Sokolowska (2016) also observed greater WSEX-Zn content with integrated use of manure and fertilizers in the surface layer. On the other hand, the P-fertilizer applied to the wheat crop showed residual effect on soil WSEX-Zn content. The WSEX-Zn content decreased with increase in level of P-fertilizer as it was higher in the treatment where no P-fertilizer was applied to the wheat crop and was the lowest where maximum P-fertilizer was applied (P_{60}). The interaction between rice and wheat was non-significant.

The mean values for SpAd-Zn ranged from 1.68 mg kg^{-1} in $M_0N_{120}P_0$ to 1.85 mg kg^{-1} in $M_6N_{80}P_{30}$ plots. The content for SpAd-Zn was highest under the treatments where manure @ 6 t ha^{-1} was applied along with N @ 80 kg ha^{-1} and P @ 30 kg ha^{-1} ($M_6N_{80}P_{30}$) and second highest in the treatment where manure @ 6 t ha^{-1} and N @ 80 kg ha^{-1} was applied without P-fertilizer ($M_6N_{80}P_0$). However, SpAd-Zn was lower under the treatments where only fertilizers were applied to the rice *i.e.* $M_0N_{120}P_0$ and $M_0N_{120}P_{30}$. The interaction between rice and wheat was non-significant.

A perusal of the data (Table 2) showed that the MnOX-Zn content (mean values) was 1.83, 1.97, 1.87 and 1.93 mg kg^{-1} for $M_0N_{120}P_0$, $M_6N_{80}P_{30}$, $M_0N_{120}P_{30}$ and $M_6N_{80}P_0$, respectively. The MnOX-Zn content was significantly higher in $M_6N_{80}P_{30}$ with respect to all other treatments ($M_0N_{120}P_0$, $M_0N_{120}P_{30}$ and $M_6N_{80}P_0$). Shuman (1999) also found an increase in Zn and Mn fractions due to organic amendments. Among the graded doses of P-fertilizer to the wheat crop, the residual effect for MnOx-Zn was found higher under P_{60} followed by P_{30} and then in P_0.

The data (Table 2) revealed that AFeOX-Zn (mean values) varied from 8.59 mg kg^{-1} in $M_0N_{120}P_0$

Table 2. Effect of manure and fertilizers on chemical fractions of Zn in soil under rice-wheat system

Treatments (Rice)	P applied to wheat (kg P_2O_5 ha^{-1}) P_0	P_{30}	P_{60}	Mean
WSEX Zn (mg kg^{-1})				
$M_0N_{120}P_0$	1.38	1.37	1.35	1.37
$M_6N_{80}P_{30}$	1.44	1.43	1.41	1.43
$M_0N_{120}P_{30}$	1.34	1.33	1.30	1.32
$M_6N_{80}P_0$	1.50	1.48	1.47	1.48
Mean	1.41	1.40	1.39	-
LSD (p<0.05)	R = 0.01, W = 0.01, R×W = NS			
SpAd Zn (mg kg^{-1})				
$M_0N_{120}P_0$	1.66	1.67	1.69	1.68
$M_6N_{80}P_{30}$	1.82	1.85	1.88	1.85
$M_0N_{120}P_{30}$	1.72	1.74	1.76	1.74
$M_6N_{80}P_0$	1.78	1.80	1.81	1.80
Mean	1.75	1.77	1.79	-
LSD (p<0.05)	R = 0.02, W = 0.008, R×W = NS			
MnOX Zn (mg kg^{-1})				
$M_0N_{120}P_0$	1.82	1.83	1.85	1.83
$M_6N_{80}P_{30}$	1.95	1.96	1.99	1.97
$M_0N_{120}P_{30}$	1.85	1.88	1.89	1.87
$M_6N_{80}P_0$	1.92	1.93	1.94	1.93
Mean	1.89	1.90	1.92	
LSD (p<0.05)	R = 0.02, W = 0.008, R×W = NS			
AFeOX Zn (mg kg^{-1})				
$M_0N_{120}P_0$	8.80	8.36	8.60	8.59
$M_6N_{80}P_{30}$	10.12	9.73	9.91	9.92
$M_0N_{120}P_{30}$	7.74	7.23	7.53	7.50
$M_6N_{80}P_0$	11.32	10.37	11.13	10.94
Mean	9.50	8.92	9.29	-
LSD (p<0.05)	R = 0.15, W = 0.12, R×W = 0.24			
CFeOX Zn (mg kg^{-1})				
$M_0N_{120}P_0$	9.46	9.32	8.98	9.26
$M_6N_{80}P_{30}$	11.12	10.94	10.03	10.70
$M_0N_{120}P_{30}$	8.87	7.98	7.34	8.06
$M_6N_{80}P_0$	12.94	12.43	11.25	12.21
Mean	10.60	10.17	9.40	-
LSD (p<0.05)	R = 0.32, W = 0.13, R×W = 0.26			
OM-bound Zn (mg kg^{-1})				
$M_0N_{120}P_0$	0.85	0.98	0.96	0.93
$M_6N_{80}P_{30}$	1.32	1.36	1.52	1.40
$M_0N_{120}P_{30}$	1.01	1.06	1.12	1.06
$M_6N_{80}P_0$	1.14	1.19	1.25	1.19
Mean	1.08	1.15	1.21	-
LSD (p<0.05)	R = 0.05, W = 0.03, R×W = 0.06			
RES Zn (mg kg^{-1})				
$M_0N_{120}P_0$	40.47	41.78	43.50	41.98
$M_6N_{80}P_{30}$	44.23	43.15	42.61	43.33
$M_0N_{120}P_{30}$	42.52	42.14	41.40	42.02
$M_6N_{80}P_0$	42.60	45.83	42.92	43.78
Mean	42.46	43.22	42.61	-
LSD (p<0.05)	R = 0.34, W = 0.41, R×W = 0.81			
Total Zn (mg kg^{-1})				
$M_0N_{120}P_0$	68.21	66.69	66.05	66.98
$M_6N_{80}P_{30}$	68.25	69.05	70.25	69.18
$M_0N_{120}P_{30}$	65.07	63.35	62.34	63.59
$M_6N_{80}P_0$	73.20	75.03	71.78	73.34
Mean	68.68	68.53	67.61	
LSD (p<0.05)	R = 0.50, W = 0.36, R×W = 0.72			

M_0 = No Manure, M_6 = Manure @ 6 t ha^{-1}, N_{80} = 80 kg N ha^{-1}, N_{120} = 120 kg N ha^{-1}, P_0 = No P_2O_5, P_{30} = 30 kg P_2O_5 ha^{-1}, P_{60} = 60 kg P_2O_5 ha^{-1}, R = Rice, W = Wheat, R×W = Interaction

to 10.94 mg kg^{-1} in $M_6N_{80}P_0$. It was observed that AFeOX-Zn was significantly higher under the treatments where manure was applied along with fertilizers *i.e.* $M_6N_{80}P_{30}$ and $M_6N_{80}P_0$. Similarly, the CFeOX-Zn content (mean values) varied as 9.26, 10.70, 8.06 and 12.21 mg kg^{-1}, respectively under $M_0N_{120}P_0$, $M_6N_{80}P_{30}$, $M_0N_{120}P_{30}$ and $M_6N_{80}P_0$ (Table 2). Similar to the AFeOX-Zn, the CFeOX-Zn was also found higher under the manure plus fertilizers treated plots as compared to the fertilizers treated plots only. The increase in AFeOX-Zn for manure plus fertilized plots over chemically fertilized plots, suggests that organic manure altered the transformation of Zn by preventing the occlusion of Zn in precipitating Fe oxides (Sims 1986). About 82% of the total soil Zn in RES fraction which was about 17% of the total Zn was associated with CFeOX fraction (Singh *et al.* 1988).

The OM-Zn (Table 2) increased significantly in soil where both the organic manure and fertilizers were applied in combination ($M_6N_{80}P_{30}$ and $M_6N_{80}P_0$) with the mean values (1.40 and 1.19 mg kg^{-1}) as compared to the plots where only fertilizers were applied. The increase in OM-Zn content in the treatments receiving fertilizer and manure application may be attributed to higher soil OC content in these treatments which increased its availability by complexing with soil OM (Umesh *et al.* 2013).

The mean value of RES-Zn content varied from 41.98 to 43.78 mg kg^{-1} and the total-Zn content from 63.59 to 73.34 mg kg^{-1} under different treatment combinations (Table 2). The highest content of RES-Zn and as total-Zn was under $M_6N_{80}P_0$ followed by $M_6N_{80}P_{30}$. The results revealed that bulk of native Zn was in the residual fraction after the total fraction as compared to other forms of Zn. The formation of organo-metalic complexes with ligands, mineralization and solubilization from organic sources might be the reason for increased concentration of Zn fractions in plots receiving organic manure. As the pH increases, the number of negative pH-dependent charges also increases. With increased in negative charge density at the surface of the colloids, the micronutrients availability for the plant decreased. Tabassum *et al.* (2014) reported increase in all fractions of Zn with application of organic manures in Vertisols of Jabalpur. However, Boguta and Sokolowska (2016) found that organic matter decreased Zn solubility by complexion of Zn with dissolved organic compounds. Similar observations were reported by Fan *et al.* (2016) and Xin *et al.* (2016).

Effect of manure and fertilizers on chemical fractions of Cu

The WSEX-Cu in the surface soil (Table 3) varied from 0.03 mg kg^{-1} in $M_6N_{80}P_{30}$ to 0.05 mg kg^{-1} in $M_6N_{80}P_0$. The WSEX-Cu content showed no significant effect within the different treatment combinations. Moreover, the content for WSEX-Cu more or less remained unchanged in different treatments. The mean value of SpAd-Cu varied from 0.02 to 0.05 mg kg^{-1} under different treatments.

The mean value of MnOX-Cu in the surface soil varied from 0.05 mg kg^{-1} in $M_0N_{120}P_0$ to 0.07 mg kg^{-1} in $M_6N_{80}P_{30}$. Application of fertilizer alone or in combination with organic manure did not significantly affect the MnOX-Cu. Highest content of MnOX-Cu was in treatment with integrated use of fertilizers and manure *i.e.* $M_6N_{80}P_{30}$. The slight increase in the content of MnOX-Cu might be due to transformation of Cu from iron oxide pools to manganese oxide bound pool. Agbenin and Henningsen (2004) also observed increase in MnOX-Cu with application of NPK and manure.

The mean value of AFeOX-Cu varied from 0.78 to 0.96 mg kg^{-1} (Table 3) and in case of CFeOX-Cu, it varied from 1.05 to 1.24 mg kg^{-1} (Table 3) under the different treatment combinations. Integrated use of organic manure and fertilizers significant increased AFeOX-Cu and CFeOX-Fe fractions as compared to the treatments where only fertilizers were applied. The interaction between rice and wheat crops was found non-significant for both AFeOX-Cu and CFeOX-Cu fractions. The higher content of Cu in AFeOX fraction in soil under rice-wheat cropping system was also observed in organically treated plots (Saha *et al*. 2000).

Results indicated that OM-Cu (Table 3) in the surface soil varied from 0.28 mg kg^{-1} in $M_0N_{120}P_0$ to 0.34 mg kg^{-1} in $M_6N_{80}P_{30}$. Likewise, the results revealed that the RES-Cu content varied from 7.01 to 8.12 mg kg^{-1} and total-Cu from 9.34 to 10.76 mg kg^{-1} under different treatment combinations. The OM-Cu, RES-Cu and total-Cu content were higher under integrated use of organic and inorganic plots ($M_6N_{80}P_{30}$ and $M_6N_{80}P_0$) as compared to the only inorganically treated plots ($M_0N_{120}P_0$ and $M_0N_{120}P_{30}$). Major part of the total-Cu content was present as residual *i.e.* held within the silicate mineral structure. The dominance of RES-Cu accords with reports that oxides in soils and clays provide reactive sites for the chemisorptions of Cu which is almost irreversible (Agbenin and Henningsen 2004). High total-Cu content in organically treated plots might be due to

Table 3. Effect of manure and fertilizers on chemical fractions of Cu in soil under rice-wheat system

Treatments (Rice)	Rates of P applied to wheat (kg P_2O_5 ha^{-1})			Mean
	P_0	P_{30}	P_{60}	
WSEX Cu (mg kg^{-1})				
$M_0N_{120}P_0$	0.04	0.04	0.04	0.04
$M_6N_{80}P_{30}$	0.03	0.04	0.04	0.04
$M_0N_{120}P_{30}$	0.04	0.04	0.04	0.04
$M_6N_{80}P_0$	0.04	0.05	0.04	0.04
Mean	0.04	0.04	0.04	-
LSD ($p<0.05$)	R = NS, W = NS, R×W = 0.006			
SpAd Cu (mg kg^{-1})				
$M_0N_{120}P_0$	0.04	0.06	0.04	0.05
$M_6N_{80}P_{30}$	0.04	0.05	0.04	0.05
$M_0N_{120}P_{30}$	0.05	0.04	0.04	0.04
$M_6N_{80}P_0$	0.02	0.04	0.01	0.02
Mean	0.04	0.05	0.04	-
LSD ($p<0.05$)	R = 0.01, W = NS, R×W = NS			
MnOX Cu (mg kg^{-1})				
$M_0N_{120}P_0$	0.05	0.06	0.06	0.05
$M_6N_{80}P_{30}$	0.08	0.06	0.06	0.07
$M_0N_{120}P_{30}$	0.07	0.05	0.05	0.06
$M_6N_{80}P_0$	0.06	0.05	0.05	0.06
Mean	0.07	0.06	0.06	-
LSD ($p<0.05$)	R = NS, W = NS, R×W = NS			
AFeOX Cu (mg kg^{-1})				
$M_0N_{120}P_0$	0.75	0.76	0.82	0.78
$M_6N_{80}P_{30}$	0.94	0.95	0.98	0.96
$M_0N_{120}P_{30}$	0.84	0.85	0.88	0.86
$M_6N_{80}P_0$	0.90	0.91	0.93	0.92
Mean	0.86	0.87	0.90	-
LSD ($p<0.05$)	R = 0.02, W = 0.01, R×W = NS			
CFeOX Cu (mg kg^{-1})				
$M_0N_{120}P_0$	1.15	1.14	1.12	1.14
$M_6N_{80}P_{30}$	1.18	1.19	1.18	1.18
$M_0N_{120}P_{30}$	1.07	1.05	1.04	1.05
$M_6N_{80}P_0$	1.26	1.23	1.22	1.24
Mean	1.16	1.15	1.14	-
LSD ($p<0.05$)	R = 0.02, W = 0.009, R×W = NS			
OM-bound Cu (mg kg^{-1})				
$M_0N_{120}P_0$	0.29	0.27	0.27	0.28
$M_6N_{80}P_{30}$	0.29	0.35	0.38	0.34
$M_0N_{120}P_{30}$	0.28	0.28	0.29	0.28
$M_6N_{80}P_0$	0.29	0.32	0.33	0.31
Mean	0.29	0.30	0.32	-
LSD ($p<0.05$)	R = 0.01, W = 0.01, R×W = 0.02			
RES Cu (mg kg^{-1})				
$M_0N_{120}P_0$	7.94	7.53	7.25	7.58
$M_6N_{80}P_{30}$	8.05	7.66	8.64	8.12
$M_0N_{120}P_{30}$	7.39	7.03	6.61	7.01
$M_6N_{80}P_0$	8.20	7.99	7.93	8.04
Mean	7.90	7.56	7.61	-
LSD ($p<0.05$)	R = 0.63, W = NS, R×W = NS			
Total Cu (mg kg^{-1})				
$M_0N_{120}P_0$	10.27	9.86	9.60	9.91
$M_6N_{80}P_{30}$	10.63	10.30	11.33	10.76
$M_0N_{120}P_{30}$	9.73	9.33	8.97	9.34
$M_6N_{80}P_0$	10.77	10.60	10.53	10.63
Mean	10.35	10.02	10.11	-
LSD ($p<0.05$)	R = 0.64, W = NS, R×W = NS			

M_0 = No Manure, M_6 = Manure @ 6 t ha^{-1}, N_{80} = 80 kg N ha^{-1}, N_{120} = 120 kg N ha^{-1}, P_0 = No P_2O_5, P_{30} = 30 kg P_2O_5 ha^{-1}, P_{60} = 60 kg P_2O_5 ha^{-1}, R = Rice, W = Wheat, R×W = Interaction build-up of organic matter under continuous manuring in soil (Chaudhary and Narwal 2005). Ojha *et al*. (2018) reported that availability of water soluble and exchangeable form of Cu decreased with time. Similar results were reported by He *et al.* (2017) and Refaey *et al.* (2017).

Conclusions

The change in Zn and Cu from one fraction to the other was induced with the addition of the manure. Availability of Zn and Cu was higher in the treatments where biogas slurry was applied along with fertilizers. The RES fraction was the dominant among all the fractions. The WSEX fraction of Zn contributes little to total Zn, as compared to the CFeOX and AFeOX fractions.

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