Original Research Article

Long Term Effect of Manure and Fertilizers on Chemical Fractions of Fe and Mn in Surface Soils under Rice-Wheat System

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ABSTRACT

The present research study has been conducted with prime objective to investigate the effect of manure and fertilizers on chemical fractions of Fe and Mn under rice-wheat system. Laboratory analysis was made on the soil samples collected (October 2013) from an on-going long-term field experiment (in progress since Kharif 2009-10) at Department of Soil Science, PAU, Ludhiana. The organic manure through bio gas slurry (BGS) @ 6 t ha⁻¹ was incorporated along with nitrogen (N @ 80 and 120 kg ha⁻¹), phosphorus (P @ 30 kg ha⁻¹) and potassium fertilizer (K @ 30 kg ha⁻¹) to the rice crop. On the other hand in the wheat crop, nitrogen (N @ 120 kg ha⁻¹), phosphorus (P @ 0, 30 and 60 kg ha⁻¹) and potassium fertilizer (K @ 30 kg ha⁻¹) were applied without addition of organic manure. It was observed that the concentration of micronutrients was found higher in the fractions where organic manure was applied along with chemical fertilizers. It was found that the residual micronutrient fraction is the dominant portion of total Fe and Mn fraction. The WSEX fraction contributed limited in amount as compared to the other fractions. Among chemical fractions viz. WSEX, SpAd, MnOX, AFeOX, CFeOX, OM-bound associated with Zn, Cu, Fe and Mn showed their edge with combined application of manure and chemical fertilizers. However, WSEX, SpAd, CFeOX and OM-bound fractions contributed towards uptake of micronutrients by wheat and rice grains.

Keywords: WSEX, SpAd, MnOX, AFeOX, CFeOX, OM-bound, RES, Fe and Mn, Biogas slurry manure, Chemical fertilizers, Rice-wheat system

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Introduction

Rice-wheat cropping system is most vital cropping system of Indian subcontinent. Rice (Oryza sativa L.) and wheat (Triticum aestivum L.) are the two most important energy giving food globally (Singh et al., 2011; Meena et al., 2013). Rice and wheat grown sequentially in an annual rotation (Singh and Singh, 2009) constitute a rice-wheat cropping system (RWCS) and in a system occupy nearly 13.5 million hectares area in the Indo-Gangetic Plains (IGP) of South Asia. Integrated nutrient management practices for rice-wheat cropping system are of supreme importance for sustainable crop production in country (Singh and Kumar, 2009). The study of various fractions of Fe
and Mn present in soil and conditions under which they become available to plants is pre-requisite in assessing their availability to plants. It is important to know the relationship between chemical fractions of micronutrients in the soil and their uptake by the crop. Under continuous cropping system, micronutrients are generally considered to be present in association with soil solution, organic and inorganic solid phases and this association is often referred to as speciation (Behera et al., 2009), thus, forming their various chemical fractions such as water soluble plus exchangeable, specifically absorbed and those associated with free calcium carbonate, oxide surfaces, soil organic matter and minerals.

The alternate flooding (reduced stage) in rice and upland (oxidized stage) conditions in wheat affects transformation of Zn and Cu from one chemical form to another (Manchanda et al., 2003). Dhaliwal (2008) reported that green manure and soil applied Mn to rice–wheat system increased the DTPA-extractable, water soluble plus exchangeable and Mn specifically adsorbed on the inorganic sites whereas, Mn held on organic sites and oxide bound surfaces decreased. Duhan and Singh (2002) reported that the use of organic manures increased uptake of micronutrients which may be attributed to increase in DTPA-extractable Zn and Fe in soil and to increased yield by these organic materials. Sekhon et al., (2006) reported that application of organic manures resulted in increase and redistribution of Zn from non-available forms to readily available (water-soluble plus exchangeable) and potentially available forms in soil.

Hellal (2007) reported that addition of composted mixtures increased MnOX-Zn in soil as a result Fe availability is increased in calcareous soil by high acidulation effect of compost. Herencia et al., (2008) showed that percentage of Zn in the specific fractions with respect to total content are Zn and addition of OM caused Zn to move from less soluble forms to more plant available fraction which was always favoured by organic amendment. Sekhon et al., (2006) reported that addition of GM to rice increased AFeOX form of Zn under rice-wheat rotation. Hellal (2007) reported that addition of composted mixtures increased amorphous Fe oxide but occluded fractions did not differ significantly due to application of composted mixtures. Consequently, the present research study was conducted with a prime objective to investigate the effect of manure and fertilizers on transformations (distribution) of micronutrients (Fe and Mn) in soil.

**Materials and Methods**

In order to achieve the objectives mentioned earlier, laboratory studies were made on the soil samples collected from an on-going long-term experiment on role of manure and fertilizers in rice-wheat cropping system (in progress since Kharif 2009-10) at Department of Soil Science, Punjab Agricultural University, Ludhiana. The soil of experiment field was classified as Typic Ustochrept. The experiment was laid out in a split plot design with four main and three sub treatments. The organic manure through bio gas slurry (BGS) @ 6 t ha\(^{-1}\) was incorporated along with nitrogen fertilizer (N @ 80 and 120 kg ha\(^{-1}\)), phosphorus fertilizer (P @ 30 kg ha\(^{-1}\)) and potassium fertilizer (K @ 30 kg ha\(^{-1}\)) were applied to the rice crop. Whereas in wheat crop, nitrogen fertilizer (N @ 120 kg ha\(^{-1}\)), different levels of phosphorus fertilizer (P @ 30 and 60 kg ha\(^{-1}\)) and potassium fertilizer (K @ 30 kg ha\(^{-1}\)) were applied (Table 1).

**Laboratory analysis**

The soil samples were used to fractionate into following chemical forms as per sequential extraction procedure described below:
Water soluble plus exchangeable fraction (WSEX)

Five grams of soil was shaken with 20 ml of 0.005 M Pb(NO₃)₂ in 100 ml centrifuge tubes for fifteen minutes at 25°C in Orbital shaker and mixture was centrifuged for ten minutes at 6000 rpm the supernatant filtered, separated and stored for analysis (Manchanda et al., 2006).

The Reagent 0.005 M Pb(NO₃)₂ is prepared by dissolving 1.65gm of lead nitrate in one litre adjusting the pH of solution to 6.8 by 0.5M ammonium acetate (NH₄OAC) which is prepared by dissolving 38.5 gm of ammonium acetate in 1 litre.

Specifically adsorbed (SpAd) fraction

The soil residue from water soluble plus exchangeable fraction was shaken with 20 ml of 0.05M Pb(NO₃)₂ for 2 hours at 25°C in orbital shaker and; the sample was, thereafter, centrifuged ten minutes at 6000 rpm and the supernatant filtered (Iwaski et al., 1993).

The sequential extraction continued in the remaining of the soil sample The Reagent 0.05 M Pb(NO₃)₂ is prepared by dissolving 16.56gm of lead nitrate in one litre adjusting the pH of solution to 6 by 0.5M ammonium acetate (NH₄OAC).

Mn-Oxide bound fraction (MnOX)

To the remaining soil sample 20.0 ml of NH₂OH.HCl (hydroxylamine hydrochloride) 0.1 mol 1⁻¹ at pH 2.0 were added and the mixture was shaken for 30 min, centrifuged and filtered; the separated supernatant was stored for analysis (Chao, 1972). The Reagent 0.1 M NH₂OH.HCl in 0.01M HNO₃ is prepared by dissolving 6.95 gm of NH₂OH.HCl and 0.625 Nitric acid (HNO₃) in water and make the volume to one litre.

Amorphous Fe-Oxides bound (AFeOX) fraction

To the Mn-Oxide Bound Fraction free soil sample 20.0 ml of NH₂OH.HCl (hydroxylamine hydrochloride) 0.1 mol 1⁻¹ plus HCl 0.25 mol 1⁻¹, at pH 1.3 were added, and the mixture was shaken for 30 min at 25°C in orbital shaker, centrifuged and filtered; the separated supernatant was stored for analysis (Maskina et al., 1998). The reagent 0.25 M NH₂OH.HCl+0.25 M HCl is prepared by dissolving 17.37 gm of NH₂OH.HCl in water and pour 21 ml of Hydrochloric acid (HCl) in it and make the volume of solution to one litre.

Crystalline Fe-Oxides bound (CFeOX) fraction

To the AFeOX free soil sample 20.0 ml of 0.25 M NH₂OH.HCl +0.25 M HCl + ascorbic acid 0.01 mol l⁻¹ at pH 1.21 were added, the mixture was heated with boiling water (100°C) in a beaker placed on hot plate for 30 minutes, shaking from time to time; there after centrifuged and filtered; the separated supernatant was stored for analysis (Manchanda et al., 2006). The sequential extraction continued in the remaining of the soil sample The Reagent 0.25 M NH₂OH.HCl +0.25 M HCl +0.1 M ascorbic acid is prepared by dissolving 17.37 gm ofNH₂OH.HCl in water, pour 21 ml of hydrochloric acid (HCl) and 17.61gm of ascorbic acid in it and make the volume of solution to one litre.

Organically bound (OM) fraction

To the CFeOX free soil sample was shaken with 20 ml of 1% Na₄P₂O₇ for one hour at 25°C in Orbital shaker and mixture was centrifuged for ten minutes at 6000 rpm the supernatant filtered, separated and stored for analysis (Raja and Iyengar, 1986). The
Reagent prepared by dissolving 4.46 gm of Sodium-pyrophosphate in one litre.

Residual (RES) fraction

Residual fraction (cation) = Total content (cation) - sum of all fractions (cation). The amount of Zn, Cu, Mn and Fe in different fractions was estimated using atomic absorption spectrophotometer.

Statistical analysis

Critical difference (CD) was used to compare the treatment effects at P<0.05. The statistical analysis was done with the help of method described (Panse and Sukhatme, 1985).

Results and Discussion

Effect of manure and fertilizers on chemical fractions of Fe

The data for WSEX-Fe presented in Table 2 of the surface soil samples which were collected after harvesting of rice ranged from 0.11 to 0.16 mg kg\(^{-1}\) in all the treatments. Significant increase in WSEX-Fe contents was observed in the treatments where organic manure @ 6 t ha\(^{-1}\) was incorporated along with N @ 80 kg ha\(^{-1}\) and P\(_2\)O\(_5\) @ 30 kg ha\(^{-1}\) and in the treatments where organic manure @ 6 t ha\(^{-1}\) was applied along with N @ 80 kg ha\(^{-1}\) without phosphatic fertilizer in contrast to the treatments where no organic manure was incorporated and only N @ 120 kg ha\(^{-1}\) was applied without P\(_2\)O\(_5\) application to the rice crop. The SPAD-Fe varied from 0.25 to 0.27 mg kg\(^{-1}\) in the treatments where organic manure and inorganic fertilizers were applied in combination and it was ranged from 0.20 to 0.24 mg kg\(^{-1}\) and 0.22 to 0.24 mg kg\(^{-1}\) in the treatments where no organic manure was incorporated and only N @ 120 kg ha\(^{-1}\) was applied without P\(_2\)O\(_5\) application to the rice crop. Iu et al., (1981) reported increase in amount of SPAD-Fe with addition of organic manure. These results are also in agreement with the results obtained by Chatterjee et al., (1992) who reported increase in this form with addition of organic manure.

The MnOX-Fe showed significant increase in its fractions with fertilizers and manure. It was reported higher in the treatments where organic and inorganic fertilizers were applied in combination as compared to the treatments where only chemical fertilizers were applied to the rice crop. The MnOX-Fe ranged from
44.77 to 48.73 mg kg\(^{-1}\) in the treatments
organic manure @ 6 t ha\(^{-1}\) was incorporated
along with N @ 80 kg ha\(^{-1}\) and P\(_2\)O\(_5\) @ 30 kg
ha\(^{-1}\) were applied and 41.58 to 43.25 mg kg\(^{-1}\)
in the treatments where organic manure @ 6
t ha\(^{-1}\) was incorporated along with N @ 80
kg ha\(^{-1}\) was applied without incorporation of
phosphatic fertilizer. On the other hand, it
varied from 36.72 to 38.35 mg kg\(^{-1}\) in the
treatments where no organic manure was
incorporated and only N @ 120 kg ha\(^{-1}\) was
applied without P\(_2\)O\(_5\) application to the rice
crop and 39.12 to 41.03 mg kg\(^{-1}\) in the
treatments where no manure was
incorporated and only chemical fertilizers
were applied. Sekhon et al., (2006) reported
that addition of organic manure to rice
increased potentially available fraction of Fe
under rice-wheat rotation. Hellal (2007)
reported that addition of composted mixtures
increased MnOX-Fe in soil, as a result Fe
availability is increased in calcareous soil by
high acidulation effect of compost.

The AFeOX-Fe ranged from 386.8 to 390.1
mg kg\(^{-1}\) in the treatments where organic
manure @ 6 t ha\(^{-1}\) was incorporated along
with N @ 80 kg ha\(^{-1}\) and P\(_2\)O\(_5\) @ 30 kg ha\(^{-1}\)
were applied. Similar pattern of increase was
observed in the treatments where organic
manure @ 6 t ha\(^{-1}\) was incorporated along
with N @ 80 kg ha\(^{-1}\) was applied without
incorporation of phosphatic fertilizer where it
ranged from 383.4 to 385.9 mg kg\(^{-1}\). Hellal
(2007) reported that addition of composted mixtures increased AFeOX fraction but
occluded Fe did not differ significantly due to
application of composted mixtures. Agbenin
(2003) reported a similar increase in AFeOX-
Fe and Mn fractions fertilized with NPK,
FYM and FYM+NPK. Singh et al., (1988) in
a study on 11 soils reported that 9 and 5 per
cent of total Fe and Mn was associated with
AFeOX fraction. The CFeOX-Fe fraction
increased in soil many folds as compared to
the other fractions (Table 3). In all the
treatments CFeOX-Zn varied from 564.80 to
631.30 mg kg\(^{-1}\) where the higher content was
noticed in the treatments where organic
manure was incorporated along with chemical
fertilizers. The significant higher
concentration ranged from 616.47 to 631.30
mg kg\(^{-1}\) of this fraction was noticed in the
treatments where organic manure @ 6 t ha\(^{-1}\)
was incorporated along with N @ 80 kg ha\(^{-1}\)
and P\(_2\)O\(_5\) @ 30 kg ha\(^{-1}\) were applied. Singh et
al., (1988) and Randhawa and Singh, (1997)
reported that about 52 per cent of the total soil
Fe was presented in RES fraction and about
41 per cent of the total Fe was associated with
CFeOX fraction. Similarly, Nayyar and
Chhibba, (2000) reported that the prevalence
of alternative oxidized and reduced conditions
under rice-wheat system caused a decline in
the content of CFeOX form concomitant with
an increase in the easily reducible AFeOX
form of these micronutrients leading to their
increased availability.

The significant increase was noticed in
organically bound fraction (OM-Fe) in the
treatments where organic manure @ 6 t ha\(^{-1}\)
was incorporated along with N @ 80 kg ha\(^{-1}\)
and P\(_2\)O\(_5\) @ 30 kg ha\(^{-1}\) were applied where it
ranged from 24.60 to 26.28 mg kg\(^{-1}\) as
compared to the treatment which were treated
inorganically and where no organic manure
was incorporated. However, the significant
difference was also observed in OM-Fe
fraction in case of wheat crop where different
levels of P\(_2\)O\(_5\) (0, 30 and 60 kg ha\(^{-1}\)) were
applied. The higher concentration of OM-Fe
and Mn in the soil solution indicated that the
micronutrients associated with the OM bound
fraction may play a beneficial role in the
uptake of these nutrients by the plants.
Sekhon et al., (2006) reported that OM bound
fraction of Fe and Mn increased with
application of organic manure in rice-wheat
system. It was observed that application of P
fertilizer and organic manure with
incorporation of straw resulted in significant
increases in soil total Cu, Zn, Fe and Mn (Li et al., 2010).

Effect of manure and fertilizers on chemical fractions of Mn

The concentration of Mn in WSEX fraction ranged from 3.77 to 4.79 mg kg\(^{-1}\) in all the treatment combinations in Table 4. Significantly increased concentration of WSEX-Mn contents was observed in the treatments where organic manure @ 6 t ha\(^{-1}\) was incorporated along with N @ 80 kg ha\(^{-1}\) and P\(_2\)O\(_5\) @ 30 kg ha\(^{-1}\) were applied where it ranged from 4.41 to 4.79 mg kg\(^{-1}\) and it ranged from 4.17 to 4.39 mg kg\(^{-1}\) in the treatments where organic manure @ 6 t ha\(^{-1}\) was incorporated along with N @ 80 kg ha\(^{-1}\) was applied without incorporation of phosphatic fertilizer as compared to the treatments where no organic manure was incorporated and only N @ 120 kg ha\(^{-1}\) was applied without P\(_2\)O\(_5\) application to the rice crop where it was ranged from 3.77 to 3.81 mg kg\(^{-1}\) and in the other treatments it varied from 3.98 to 4.27 mg kg\(^{-1}\) where no manure was incorporated and only chemical fertilizers like N @ 120 kg ha\(^{-1}\) and P\(_2\)O\(_5\) @ 30 kg ha\(^{-1}\) were applied to the rice crop. The maximum concentration of WSEX-Mn was reported in organically treated plots which may be attributed to the reduction of higher valent forms of Mn (Mn\(^{4+}\)) to its available form (Mn\(^{2+}\)) accompanied by increase in its solubility under submerged conditions and chelating action of the organic manures. Earlier authors have reported that balanced fertilization not only increases grain yield and maintains soil nutrient balance, but also accelerates rice nutrient uptake (Mann et al., 2006; Li et al., 2007; Xue et al., 2014).

The SPAD-Mn reported significantly higher magnitude in the treatments where organic manure was incorporated in combination with chemical fertilizers in contrast to the treatments where no organic manure was incorporated and only chemical fertilizers were applied. The SPAD-Mn varied from 2.25 to 2.89 mg kg\(^{-1}\) in all the treatments. Significant higher concentrations were reported in organically treated plots. Lu et al., (1981) reported increase in amount of SPAD-Mn with the addition of organic manure. These results are also in agreement with the results obtained by Chatterjee et al., (1992) who reported increase in this form with addition of organic manure. Dhaliwal (2008) reported that rice-wheat cropping system increased the levels of Mn in WSEX and SPAD on the inorganic sites, whereas Mn held on organic sites and oxide bound surfaces decreased.

The MnOX-Mn showed significant increase in its fractions with fertilizers and manure. It was reported higher in the treatments where organic and inorganic fertilizers were applied in combination as compared to the treatments where only chemical fertilizers were applied to the rice crop. The MnOX-Mn ranged from 61.43 to 66.20 mg kg\(^{-1}\) in the treatments organic manure @ 6 t ha\(^{-1}\) was incorporated along with N @ 80 kg ha\(^{-1}\) and P\(_2\)O\(_5\) @ 30 kg ha\(^{-1}\) were applied and 68.20 to 73.90 mg kg\(^{-1}\) in the treatments where organic manure @ 6 t ha\(^{-1}\) was incorporated along with N @ 80 kg ha\(^{-1}\) was applied without incorporation of phosphatic fertilizer. On the other hand, it varied from 52.87 to 56.63 mg kg\(^{-1}\) in the treatments where no organic manure was incorporated and only N @ 120 kg ha\(^{-1}\) was applied without P\(_2\)O\(_5\) application to the rice crop and 50.97 to 55.40 mg kg\(^{-1}\) in the treatments where no manure was incorporated and only chemical fertilizers were applied. Sekhon et al., (2006) reported that addition of organic manure to rice increased potentially available fraction of Mn under rice-wheat rotation. Hellal (2007) reported that addition of composted mixtures increased MnOX-Mn in soil, as a result Fe availability is increased.
in calcareous soil by high acidulation effect of compost. Herencia et al., (2008) showed the percentage of Fe and Mn in the specific fractions with respect to the total content are Mn>Fe and addition of organic matter caused Zn and Fe to move from less soluble forms to more plant available fraction which was always favoured by organic amendments.

The AFeOX-Mn reported significantly higher concentration in the treatments where organic manure and chemical fertilizers were applied in combination to the rice crop. Among the treatments, organically treated plots showed higher release of AFeOX-Mn in solution. The AFeOX-Mn ranged from 22.40 to 23.67 mg kg$^{-1}$ in the treatments where organic manure @ 6 t ha$^{-1}$ was incorporated along with N @ 80 kg ha$^{-1}$ and P$_2$O$_5$ @ 30 kg ha$^{-1}$ were applied. Similar increase was observed in the treatments where organic manure @ 6 t ha$^{-1}$ was incorporated along with N @ 80 kg ha$^{-1}$ was applied without incorporation of phosphatic fertilizer where it ranged from 20.03 to 21.87 mg kg$^{-1}$. However, it varied from 15.77 to 16.53 mg kg$^{-1}$ in the treatments where no organic manure was incorporated and only N @ 120 kg ha$^{-1}$ was applied without P$_2$O$_5$ application to the rice crop and 17.30 to 21.87 mg kg$^{-1}$ in the other treatments where no manure was incorporated and only chemical fertilizers like N @ 120 kg ha$^{-1}$ and P$_2$O$_5$ @ 30 kg ha$^{-1}$ were applied. However, the significant difference was also observed in AFeOX-Mn fraction in the wheat crop where different levels of P$_2$O$_5$ (0, 30 and 60 kg ha$^{-1}$) were applied. The interaction between rice and wheat crops was found as non significant. Sekhon et al., (2006) reported that addition of organic manure to rice increased AFeOX form of Mn under rice-wheat rotation. Agbenin (2003) reported a similar increase in AFeOX-Mn fractions fertilized with NPK, FYM and FYM+NPK. Singh et al., (1988) in a study on 11 soils reported that 9 and 5 per cent of total Fe and Mn was associated with AFeOX fraction. The significantly higher concentration of CFeOX-Mn fraction was reported in the treatments where organic manure was incorporated along with chemical fertilizers (Table 5).

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

| Treatments | Manure (t ha$^{-1}$) | Rice N (kg ha$^{-1}$) | P$_2$O$_5$ (kg ha$^{-1}$) | Wheat P$_2$O$_5$ (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                            |
### Table 2: Chemical fractions of Fe (WSEX, SpAd, MnOX and AFeOX) in surface soil (0-15cm) under rice-wheat system

| Treatments of rice | Rates of P applied to wheat (kg P₂O₅ ha⁻¹) | Mean |  
|--------------------|---------------------------------------------|------|  
|                   | P₀   | P₃₀ | P₆₀ |  
| WSEX-Fe (mg kg⁻¹) |      |     |     |  
| M₀ N₁₂₀ P₀        | 0.11 | 0.12| 0.12| 0.11  
| M₆ N₈₀ P₃₀        | 0.15 | 0.15| 0.16| 0.15  
| M₀ N₁₂₀ P₃₀       | 0.11 | 0.11| 0.12| 0.11  
| M₆ N₈₀ P₀         | 0.11 | 0.13| 0.15| 0.13  
| Mean               | 0.12 | 0.13| 0.14| -     
| LSD (p<0.05)      | R=0.01, W=NS, RxW=NS |  

| SpAd-Fe (mg kg⁻¹) |      |     |     |  
| M₀ N₁₂₀ P₀        | 0.22 | 0.20| 0.24| 0.22  
| M₆ N₈₀ P₃₀        | 0.25 | 0.25| 0.27| 0.26  
| M₀ N₁₂₀ P₃₀       | 0.24 | 0.22| 0.23| 0.23  
| M₆ N₈₀ P₀         | 0.26 | 0.25| 0.27| 0.26  
| Mean               | 0.24 | 0.23| 0.25| -     
| LSD (p<0.05)      | R=0.02, W=0.009, RxW=0.02 |  

| MnOX-Fe (mg kg⁻¹) |      |     |     |  
| M₀ N₁₂₀ P₀        | 36.72| 37.84| 38.35| 37.64  
| M₆ N₈₀ P₃₀        | 44.77| 45.60| 48.73| 46.37  
| M₀ N₁₂₀ P₃₀       | 39.12| 41.03| 40.78| 40.31  
| M₆ N₈₀ P₀         | 42.86| 41.58| 43.25| 42.56  
| Mean               | 40.87| 41.51| 42.78| -     
| LSD (p<0.05)      | R=1.62, W=1.15, RxW=NS |  

| AFeOX-Fe (mg kg⁻¹) |      |     |     |  
| M₀ N₁₂₀ P₀        | 366.5| 368.5| 376.8| 370.6  
| M₆ N₈₀ P₃₀        | 386.8| 389.9| 390.1| 388.9  
| M₀ N₁₂₀ P₃₀       | 377.1| 378.5| 382.0| 379.2  
| M₆ N₈₀ P₀         | 385.9| 383.4| 385.1| 384.8  
| Mean               | 379.1| 380.1| 383.5| -     
| LSD (p<0.05)      | R=1.55, W=0.85, RxW=1.70 |
Table 3 Chemical fractions of Fe (CFeOX, OM, RES and Total) in surface soil (0-15cm) under rice-wheat system

| Treatments of rice | Rates of P applied to wheat (kg P₂O₅ ha⁻¹) | Mean |
|--------------------|-------------------------------------------|------|
|                    | P₀ | P₃₀ | P₆₀ |               |
| CFeOX-Fe (mg kg⁻¹) |    |     |     |               |
| M₀ N₁₂₀ P₀         | 564.8 | 570.2 | 569.5 | 568.2 |
| M₆ N₈₀ P₃₀         | 616.5 | 626.7 | 631.3 | 624.8 |
| M₀ N₁₂₀ P₃₀        | 579.8 | 582.3 | 585.0 | 582.4 |
| M₆ N₈₀ P₀          | 587.8 | 598.4 | 607.4 | 597.9 |
| Mean               | 587.2 | 594.4 | 598.3 | -   |
| LSD (p<0.05)       | R=1.80, W=1.32, RxW=NS                    |

| OM-bound Fe (mg kg⁻¹) |    |     |     |       |
|----------------------|----|-----|-----|-------|
| M₀ N₁₂₀ P₀           | 22.62 | 22.84 | 24.68 | 23.38 |
| M₆ N₈₀ P₃₀           | 24.60 | 26.28 | 26.02 | 25.63 |
| M₀ N₁₂₀ P₃₀          | 23.78 | 24.58 | 24.71 | 24.36 |
| M₆ N₈₀ P₀            | 24.99 | 25.09 | 24.65 | 24.91 |
| Mean                 | 23.99 | 24.70 | 25.02 | -     |
| LSD (p<0.05)         | R=1.15, W=0.64, RxW=NS                    |

| RES-Fe (%) |    |     |     |       |
|------------|----|-----|-----|-------|
| M₀ N₁₂₀ P₀ | 1.33 | 1.30 | 1.28 | 1.30 |
| M₆ N₈₀ P₃₀ | 1.37 | 1.36 | 1.34 | 1.36 |
| M₀ N₁₂₀ P₃₀ | 1.29 | 1.26 | 1.25 | 1.26 |
| M₆ N₈₀ P₀  | 1.41 | 1.39 | 1.38 | 1.39 |
| Mean       | 1.35 | 1.33 | 1.31 | -     |
| LSD (p<0.05)| R=0.01, W=0.01, RxW=NS                  |

| Total-Fe (%) |    |     |     |       |
|--------------|----|-----|-----|-------|
| M₀ N₁₂₀ P₀  | 1.43 | 1.40 | 1.38 | 1.40 |
| M₆ N₈₀ P₃₀  | 1.47 | 1.47 | 1.45 | 1.46 |
| M₀ N₁₂₀ P₃₀ | 1.39 | 1.36 | 1.35 | 1.37 |
| M₆ N₈₀ P₀   | 1.51 | 1.49 | 1.48 | 1.49 |
| Mean        | 1.45 | 1.43 | 1.42 | -     |
| LSD (p<0.05)| R=0.01, W=0.01, RxW=NS                  |
Table 4 Chemical fractions of Mn (WSEX, SpAd, MnOX and AFeOX) in surface soil (0-15cm) under rice-wheat system

| Treatments of rice | Rates of P applied to wheat (kg P$_2$O$_5$ ha$^{-1}$) | Mean      |
|--------------------|------------------------------------------------------|-----------|
|                    | P$_0$ | P$_{30}$ | P$_{60}$ |
| WSEX-Mn (mg kg$^{-1}$) |
| M$_6$ N$_{120}$ P$_0$ | 3.99  | 3.77     | 3.81     | 3.86     |
| M$_6$ N$_{80}$ P$_{30}$ | 4.41  | 4.79     | 4.64     | 4.61     |
| M$_6$ N$_{120}$ P$_{30}$ | 4.01  | 3.98     | 4.27     | 4.08     |
| M$_6$ N$_{80}$ P$_0$    | 4.17  | 4.25     | 4.39     | 4.27     |
| Mean                  | 4.14  | 4.20     | 4.28     | -        |
| LSD (p<0.05)          |       | R=0.41, W=NS, RxW=NS |
| SpAd-Mn (mg kg$^{-1}$) |
| M$_0$ N$_{120}$ P$_0$  | 2.25  | 2.36     | 2.47     | 2.36     |
| M$_6$ N$_{80}$ P$_{30}$ | 2.77  | 2.80     | 2.89     | 2.82     |
| M$_6$ N$_{120}$ P$_{30}$ | 2.55  | 2.67     | 2.76     | 2.66     |
| M$_6$ N$_{80}$ P$_0$   | 2.85  | 2.91     | 2.69     | 2.82     |
| Mean                  | 2.60  | 2.69     | 2.70     | -        |
| LSD (p<0.05)          |       | R=0.20, W=NS, RxW=NS |
| MnOX-Mn (mg kg$^{-1}$) |
| M$_0$ N$_{120}$ P$_0$  | 52.87 | 54.80    | 56.63    | 54.77    |
| M$_6$ N$_{80}$ P$_{30}$ | 61.43 | 62.43    | 66.20    | 63.36    |
| M$_6$ N$_{120}$ P$_{30}$ | 50.97 | 55.40    | 54.00    | 53.46    |
| M$_6$ N$_{80}$ P$_0$   | 68.20 | 68.80    | 73.90    | 70.30    |
| Mean                  | 58.37 | 60.36    | 62.68    | -        |
| LSD (p<0.05)          |       | R=1.23, W=0.81, RxW=1.62 |
| AFeOX-Mn (mg kg$^{-1}$) |
| M$_0$ N$_{120}$ P$_0$  | 15.77 | 16.13    | 16.53    | 16.14    |
| M$_6$ N$_{80}$ P$_{30}$ | 22.40 | 22.53    | 23.67    | 22.87    |
| M$_6$ N$_{120}$ P$_{30}$ | 17.30 | 19.23    | 19.63    | 18.72    |
| M$_6$ N$_{80}$ P$_0$   | 20.30 | 20.03    | 21.87    | 20.73    |
| Mean                  | 18.94 | 19.48    | 20.43    | -        |
| LSD (p<0.05)          |       | R=1.08, W=1.16, RxW=NS |
| Treatments of rice | Rates of P applied to wheat (kg P₂O₅ ha⁻¹) | Mean |
|--------------------|--------------------------------------------|------|
|                    | P₀  | P₃₀ | P₆₀ |
| CFeOX-Mn (mg kg⁻¹) |     |     |     |
| M₀ Nₑ₂₀ P₀         | 19.47 | 19.33 | 20.14 | 19.65 |
| M₆ N₈₀ P₃₀        | 20.20 | 20.27 | 20.70 | 20.39 |
| M₀ N₁₂₀ P₃₀       | 16.70 | 17.13 | 18.40 | 17.41 |
| M₆ N₈₀ P₀         | 20.50 | 20.90 | 21.67 | 21.02 |
| Mean               | 19.22 | 19.41 | 20.23 |      |
| LSD (p<0.05)       |      |      |      | R=1.11, W=0.70, RxW=NS |

|                   | OM-bound-Mn (mg kg⁻¹) |      |
|--------------------|------------------------|------|
| M₀ Nₑ₂₀ P₀         | 1.26                   | 1.28 |
| M₆ N₈₀ P₃₀        | 1.44                   | 1.55 |
| M₀ N₁₂₀ P₃₀       | 1.30                   | 1.35 |
| M₆ N₈₀ P₀         | 1.37                   | 1.43 |
| Mean               | 1.35                   | 1.41 |
| LSD (p<0.05)       |                       |      | R=0.07, W=0.05, RxW=NS |

|                   | RES-Mn (mg kg⁻¹)      |      |
|--------------------|-----------------------|------|
| M₀ Nₑ₂₀ P₀         | 93.20                 | 92.45 |
| M₆ N₈₀ P₃₀        | 84.27                 | 81.24 |
| M₀ N₁₂₀ P₃₀       | 95.28                 | 87.09 |
| M₆ N₈₀ P₀         | 84.11                 | 77.37 |
| Mean               | 89.22                 | 84.54 |
| LSD (p<0.05)       |                       |      | R=2.07, W=2.80, RxW=NS |

|                   | Total-Mn (mg kg⁻¹)    |      |
|--------------------|-----------------------|------|
| M₀ Nₑ₂₀ P₀         | 192.1                 | 190.1 |
| M₆ N₈₀ P₃₀        | 196.9                 | 195.5 |
| M₀ N₁₂₀ P₃₀       | 188.1                 | 186.8 |
| M₆ N₈₀ P₀         | 201.5                 | 198.9 |
| Mean               | 194.7                 | 192.9 |
| LSD (p<0.05)       |                       |      | R=0.30, W=0.48, RxW=NS |

The significant higher concentration that ranged from 20.20 to 20.70 mg kg⁻¹ of this fraction was noticed in the treatments where organic manure @ 6 t ha⁻¹ was incorporated along with N @ 80 kg ha⁻¹ and P₂O₅ @ 30 kg ha⁻¹ were applied. Similar increase was observed in the treatments where organic manure @ 6 t ha⁻¹ was incorporated along with N @ 80 kg ha⁻¹ was applied without incorporation of phosphatic fertilizer where it ranged from 20.50 to 21.67 mg kg⁻¹. The higher concentrations of CFeOX-Mn reported that Mn requirement can be maintained from this fraction as Mn associated with CFeOX released more concentration of Mn in the soil solution. Singh et al., (1988) in a study on 11 soils reported that 11 and 17 per cent of total Mn were associated with CFeOX fraction.
The significant increase was noticed in organically bound fraction (OM-Mn) in the treatments where organic manure was incorporated along with chemical fertilizers were applied where it ranged from 1.44 to 1.55 mg kg\(^{-1}\) and 1.37 and 1.43 mg kg\(^{-1}\) as compared to the treatment which were treated inorganically and it ranged from 1.26 to 1.30 mg kg\(^{-1}\) and 1.30 to 1.35 mg kg\(^{-1}\), also no organic manure was incorporated in these treatments. However, the significant difference was also observed in OM-Mn fraction in case of wheat crop where different levels of P\(_2\)O\(_5\) (0, 30 and 60 kg ha\(^{-1}\)) were applied. The interaction between rice and wheat crops was found as non-significant. The higher concentration of OM-Mn in the soil solution indicated that the micronutrients associated with the OM bound fraction may play a beneficial role in the uptake of these nutrients by the plants.

Sekhon et al., (2006) reported that OM bound fraction of Mn increased with application of organic manure in rice-wheat system.

The RES-Mn varied from 71.05 to 93.20 mg kg\(^{-1}\) in all the treatments. The concentration for RES-Mn was observed higher as compared to all other fractions except total Mn fraction. The higher level of Mn in these fractions under rice-wheat cropping system may be due to effect of submergence.

In conclusion, the inter conversion of Fe and Mn from one fraction to the other was accelerated with the addition of the manure. So, the concentration of Fe and Mn were found higher in the treatments where biogas slurry was incorporated along with inorganic fertilizers in different fractions viz. WSEX, SpAd, MnOX, AFeOX, CFeOX, OM-bound, RES and Total. The residual (RES) fraction is the dominating fraction among all the different fractions. Water soluble and exchangeable (WSEX) fraction contributes little as compared to the other fractions viz. Crystalline Fe-oxide (CFeOX) and Amorphous Fe-oxide (AFeOX) fractions. The organic compounds released during decomposition of manures enhanced the availability of Fe and Mn by preventing fixation, oxidation, precipitation and leaching.

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