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Long Term Effect of Manure and Fertilizers on Depthwise Distribution of Total Zn, Cu, Fe and Mn under Rice-Wheat System

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ABSTRACT

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 to investigate the effect of manure and chemical fertilizers in rice (*Oryza sativa* L.) - wheat (*Triticum aestivum* L.) system. 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}) to the rice crop. The results of our study reported that the total Zn decreased with increase in soil depth, whereas the increased concentration of total Cu, Fe and Mn was observed at lower soil depths. Higher concentration of total Zn, Cu, Fe and Mn was also found in the treatments where organic manure (BGS) was added @ 6 t ha^{-1} along with N @ 80 kg ha^{-1} and K @ 30 kg ha^{-1} to the rice crop. The results of this study were of practical utility since application of manure and chemical fertilizers together increased total Zn, Cu, Fe and Mn in the surface soil whereas, their concentrations decreased with depth.

Introduction

Rice (*Oryza sativa* L.) - wheat (*Triticum aestivum* L.) is the predominant cropping system, being practiced by majority of farmers in different agro-climatic zones of Punjab. Production of food grains is increasing year after year due to intensive cultivation of land thereby depleting a huge amount of macronutrients along with micronutrients. Relatively, over use of macronutrient fertilizers, decreased use of organic manures, reduced recycling of crop residues, and bumper harvests in the past three decades have induced secondary and micronutrient deficiencies in the Indo Gangetic Plains. Dhaliwal and Walia (2008) reported that incorporation of manures in the soil has beneficial effect on soil health by improving physico-chemical properties besides supplying the micronutrients like Zn, Cu, Fe and Mn. Herencia *et al.,* (2008) reported that with the addition of organic and mineral fertilization, OM-bound fractions of micronutrients increased their availability and uptake in the soil. Singh*et al.,* (1988) reported that Fe and Mn associated with organic matter (OM-bound fraction) increased with application of manure, which increased the yield under rice-wheat system. However, Sharma *et al.,*(2004) reported the decrease of total fraction with green manure after the harvest of wheat which could be due to an increase in the water soluble plus exchangeable fraction as well as held on inorganic sites. In a field experiment, Behera *et al.,* (2008) reported the distribution of total micronutrient, Zn fractions and their contribution toward availability and plant uptake of Zn under long-term maize-wheat cropping in an inceptisol. Dhaliwal *et al.,* (2011) reported distribution of total micronutrient fractions and their contribution towards availability and plant uptake of micronutrient under long term maize-wheat cropping sequence indicated residual micronutrients as the dominant proportion of total Zn and Cu. Zhang *et al.,* (2008) observed the long term effect of manure application on micronutrients availability under rice-wheat and maize-wheat cropping systems. They reported that the DTPA-extractable Zn and Cu availability was higher in maize-wheat cropping system as compared to rice-wheat cropping system. Distribution of total Zn, Cu, Fe and Mn and their contribution towards availability and plant uptake under long-term maize-wheat system was reported by Agbenin and Henningsen (2003) who indicated that the residual micronutrient fraction as the dominant portion of total Zn, Cu, Fe, and Mn fraction. Similarly, Behera *et al.,* (2008 & 2009) reported the distribution of total Zn fraction and their contribution toward availability and plant uptake of Zn under long-term maize-wheat cropping in an Inceptisol.

Singh *et al.,* (1988) reported the distribution of total Zn, Cu, Fe and Mn and their fractions in soils. In sequential extraction scheme which fractionated Zn, Cu, Fe and Mn into exchangeable, carbonates-bound, organically bound, Mn-oxide-bound, amorphous Fe-oxide bound, crystalline Fe-oxide bound, residual forms. It has been further reported that about 82, 62, 52 and 53 % of the total soil Zn, Cu, Fe, and Mn respectively, was associated with residual fraction whereas, 17, 17, 41 and 11% of the total Zn, Cu, Fe and Mn respectively was associated with the crystalline Fe-oxide bound fraction. 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 due to their conversion to other forms (Dhaliwal *et al*., 2008). The vertical distribution of micronutrients was observed by Verma *et al.,* (2005a and 2005b) in different soil profiles on different physiographic units in central Punjab which reported higher content of micronutrient in fine textured soils of old flood plain and lower content in sandy soils. They further reported that DTPA -Zn, Cu and Fe decreased with increase in soil depth but DTPA - Mn did not follow a definite trend.

Various research studies revealed that application of manure in combination with chemical fertilizers increased the OC level and available macro as well as micronutrients in soil. The information on surface and depth wise distribution of available micronutrients with application of manure and fertilizers is meager in rice-wheat system. Also the information of micronutrient fractions in the system is lacking and the effect of manure and fertilizers on physico-chemical properties of soil under this system needs to be investigated. Taking these points into consideration the research has been conducted to study the depth wise variation of total Zn, Cu, Fe and Mn in soils under rice-wheat system.

Materials and Methods

Experimental site and treatment details

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 conducted in a fixed layout since its beginning with treatments combinations mentioned in Table 1. 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 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 phosphatic fertilizer (P @ 30 and 60 kg ha^{-1}) and potassium fertilizer (K @ 30 kg ha^{-1}) were applied.

Various physico-chemical properties of the experimental soil are given in Table 2. The pH of the soil was 6.01 and the EC was 0.17 dS m^{-1}. The soil organic carbon was 0.33%, available nitrogen content was 275 kg ha^{-1}, available phosphorus was 23 kg ha^{-1} and available potassium was observed as 184 kg ha^{-1}. The concentration of Zn, Cu, Fe and Mn was noted as 1.76, 0.67, 5.87 and 4.59 mg kg^{-1}.

Treatment details

The experiment consists of 12 treatments with three replications under split plot design (Table 1). Biogas manure was applied @ 6 t ha^{-1} before transplantation of rice with different combinations of nitrogen and phosphorus fertilizers were applied to rice and wheat crops. Similarly, profile samples from six periodic depths (0-15, 15-30, 30-60, 60-90, 90-120, 120-150 cm) were collected after harvesting of rice in the month of October, 2013. Soil samples were analyzed for DTPA-extractable and total Zn, Cu, Fe and Mn.

Laboratory analysis

Total Zn, Cu, Fe and Mn

For total elemental analysis of Zn, Cu, Fe and Mn, a 0.5 gm sample of soil was digested with 5 ml of hydrofluoric acid (HF), 1.0 ml of perchloric acid ($HClO_4$) and 5-6 drops of nitric acid (HNO_3) in a 30 ml capacity platinum crucibles (Page *et al*., 1982). When the soil became completely dry in the crucible the residue in the crucible was completely dissolved in 5ml of 6N HCl.

The contents of the crucible were transferred to 100 ml volumetric flask with double distilled water. The digests were analyzed for total Zn, Cu, Fe and Mn after appropriate dilutions. The results of the elemental analysis were reported on an oven-dry weight basis.

Laboratory analysis

Different parameters pertaining to analysis of soil were subjected to split plot block design analysis of variance. 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 by Panse and Sukhatme (1985).

Results and Discussion

The soil samples collected after harvesting of rice from an ongoing long term experiment were subjected to estimation for total micronutrients viz. Zn, Cu, Fe and Mn.

Depthwise distributions of total Zn

The data presented in Table 3 showed the distribution of total Zn in profile soil (0-150 cm) samples collected after harvesting of rice crop. The data revealed that the higher concentrations of total Zn were observed in surface soil as compared to subsurface soil. Normally the total Zn decrease with increase in soil depth under rice-wheat cropping system.

The content of total Zn in surface soil (0-15 cm) ranged from 46.47 to 55.70 mg kg^{-1} in all the treatments. It was found significantly higher in surface soil where organic manure has been added along with the chemical fertilizers whereas in subsurface layers at 15-30, 30-60, 60-90, 90-120 and 120-150 cm soil depths, the concentrations of total Zn decreased, ranged from 27.87 to 36.30, 20.60 to 29.37, 20.10 to 26.97, 21.07 to 28.27 and 22.17 to 29.47 mg kg^{-1} which may be due to higher organic matter present in surface soil as compare to subsurface soil.

The concentration decreases upto 90-120 cm soil depth and then it increased at 120-150 cm soil depth. Significantly higher concentration of total Zn was observed in the treatments where organic manure @ 6 t ha^{-1} has been incorporated along with N @ 80 kg ha^{-1} and P_2O_5 @ 30 kg ha^{-1} applied to the rice crop as compared to the treatments where only N @ 120 kg ha^{-1} was applied without application of organic manure and P_2O_5 to the rice crop. Similarly, significant increased concentration of total Zn was also noticed in the treatments where organic manure @ 6 t ha^{-1} was incorporated along with N @ 80 kg ha^{-1} without the application of phosphatic fertilizer as compared to the treatments N @ 120 kg ha^{-1} and P_2O_5 @ 30 kg ha^{-1} were applied to the rice crop without addition of organic manure. The significant increase in the concentration of total Zn in the treatments where organic manure @ 6 t ha^{-1} was added along with N @ 80 kg ha^{-1} and P_2O_5 @ 30 kg ha^{-1} applied to the rice crop as compared to the treatments where no organic manure was incorporated, only N @ 120 kg ha^{-1} and P_2O_5 @ 30 kg ha^{-1} were applied. Whereas in the wheat crop, the different levels of P_2O_5 (0, 30 and 60 kg ha^{-1}) were applied, significant results in concentration of total Zn has been observed with increase in levels of phosphatic fertilizer. The interaction between rice and wheat crop is observed as non significant.

Depth wise distribution of total Cu

Higher concentration of total Cu was observed in the surface soil samples under the rice-wheat cropping system as compared to the subsurface soil samples (Table 4). The concentration of total Cu ranged from 7.20 to 9.43 mg kg^{-1} in surface soil (0-15 cm). The total Cu increased with increased in soil depth but its concentration start decreasing with increase in depth. The concentration for total Cu ranged from 7.68 to 10.32, 8.43 to 9.87, 8.97 to 9.93, 8.77 to 9.87 and 8.73 to 9.93 mg kg^{-1} in 15-30, 30-60, 60-90, 90-120 and 120-150 cm soil depth, respectively. Among the different treatments, a significant increase in total Cu was noticed in the treatments where organic manure @ 6 t ha^{-1} has been incorporated along with N @ 80 kg ha^{-1} and P_2O_5 @ 30 kg ha^{-1} to the rice crop as compared to the treatments where only N @ 120 kg ha^{-1} was applied without P_2O_5 and organic manure application to the rice crop. Significantly higher concentration of total Cu was also noticed in the treatments where organic manure @ 6 t ha^{-1} was applied along with N @ 80 kg ha^{-1} without the application of phosphatic fertilizer as compared to the treatments where N @ 120 kg ha^{-1} and P_2O_5 @ 30 kg ha^{-1} were applied without organic manure addition to the rice crop. In the wheat crop, where the different levels of P_2O_5 (0, 30 and 60 kg ha^{-1}) were applied, a significant response has been observed in concentration of total Cu at these levels of phosphatic fertilizer. The interaction between rice and wheat treatments was also observed as significant. This increase in availability of total Cu in the plots which were treated organically along with chemical fertilizers may be due to reduction in the redox - potential of the soil with the addition of organic manures which lead to more release of micronutrients in an available form in the soil as compared to the application of chemical fertilizer alone. It was observed that total Cu remained insignificant at lower soil depths and the interaction between the two treatments also found to be non significant.

Depthwise distribution of total Fe

The data presented in Table 5 showed the distribution of total Fe in soil profile (0-150 cm) samples collected after harvesting of rice. The results revealed that concentrations of total Fe ranged from 1.25 to 1.51 per cent in surface soil (0-15 cm) and these concentrations further increased with increase in soil depths. It was observed that the total Fe concentration varied from 1.40 to 1.55, 1.45 to 1.65, 1.57 to 1.73, 1.56 to 1.74 and 1.64 to 1.81 % in 15-30, 30-60, 60-90, 90-120 and 120-150 cm soil depth, respectively.

Significantly higher level of total Fe was reported in the treatments where organic manure @ 6 t ha^{-1} has been incorporated along with N @ 80 kg ha^{-1} and P_2O_5 @ 30 kg ha^{-1} to the rice crop as compared to the treatments where N @ 120 kg ha^{-1} was applied without organic manure and P_2O_5 application to the rice crop. Significantly higher concentration of total Fe was also noticed in the treatments where organic manure @ 6 t ha^{-1} was added along with N @ 80 kg ha^{-1} without the application of phosphatic fertilizer as compared to the treatments where N @ 120 kg ha^{-1} and P_2O_5 @ 30 kg ha^{-1} were applied to the rice crop without addition of organic manure. On the other hand in the wheat crop, where the different levels of P_2O_5 (0, 30 and 60 kg ha^{-1}) were applied, a significant response has been observed in concentration of total Fe at these levels of phosphatic fertilizer. Higher content of total Fe was observed which may be due to the effect of submergence and may be further associated with more organic matter present in surface soil. Similar results were observed by Khan *et al*., (2002), who reported the higher concentration of total Fe in surface (0-15 cm) as compared to the subsurface layers under rice-wheat system. Similarly, Elbordiny and Camilia (2008) reported that the significantly positive correlation of total Fe with organic matter content in surface and subsurface soil. The interaction between rice and wheat crop treatments is also observed as significant.

Depthwise distributions of total Mn

The total Mn concentration in surface soil (0-15 cm) ranged from 203.6 to 219.8 mg kg^{-1} under rice-wheat cropping system (Table 6). The total Mn increased with increase in soil depth. It varied from 285.5 to 305.3, 297.2 to 326.4, 316.5 to 345.3, 332.7 to 360.9 and 363.6 to 386.5 mg kg^{-1} at 15-30, 30-60, 60-90, 90-120 and 120-150 cm soil depths, respectively. The level of total Mn at lower depths was higher may be due to submergence and it leached down to lower layers. The data reported the significant increase in the concentration of total Mn in the treatments where organic manure @ 6 t ha^{-1} has been added along with N @ 80 kg ha^{-1} and P_2O_5 @ 30 kg ha^{-1} which were applied to the rice crop as compared to the treatments where only N @ 120 kg ha^{-1} was applied without organic manure and P fertilizer application.

Significantly higher concentration of total Mn was also noticed in the treatments where organic manure @ 6 t ha^{-1} was incorporated along with N @ 80 kg ha^{-1} without the application of phosphatic fertilizer as compared to the treatments where no organic manure was added but N @ 120 kg ha^{-1} and P_2O_5 @ 30 kg ha^{-1} were applied to the rice crop. Also, the significantly higher concentration was observed in the treatments where organic manure @ 6 t ha^{-1} was added along with N @ 80 kg ha^{-1} and P_2O_5 @ 30 kg ha^{-1} applied to the rice crop as compared to the treatments where no organic manure was incorporated but N @ 120 kg ha^{-1} and P_2O_5 @ 30 kg ha^{-1} were applied to the rice crop. Whereas in the wheat crop, the different levels of P_2O_5 (0, 30 and 60 kg ha^{-1}) were applied, a significant response was observed in concentration of total Mn at these levels.

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

Treatments	Rice			Wheat
	Manure	N	P_2O_5	P_2O_5
	(t ha^{-1})	(kg ha^{-1})		(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 Physico-chemical properties of experimental soil before sowing of wheat

Parameter	Value
Texture	Loamy sand
pH (1:2)	6.71
EC (dSm^{-1})	0.17
Organic carbon (%)	0.33
Available nitrogen (kg ha^{-1})	275
Available phosphorus (kg ha^{-1})	23
Available potassium (kg ha^{-1})	184
Total zinc (mg kg^{-1})	26.46
Total copper (mg kg^{-1})	6.62
Total iron (%)	1.14
Total manganese (mg kg^{-1})	186.4

Table.3 Depth wise distribution of total Zn (mg kg^{-1}) under rice-wheat system

Treatments of rice	Rates of P applied to wheat (kg P_2O_5 ha^{-1})			Mean	Rates of P applied to wheat (kg P_2O_5 ha^{-1})			Mean
	P_0	P_{30}	P_{60}		P_0	P_{30}	P_{60}	
0-15 cm					**60-90 cm**			
M_0 N_{120} P_0	51.37	50.60	50.93	50.97	23.40	22.10	21.20	22.23
M_6 N_{80} P_{30}	53.03	53.40	50.67	52.37	24.33	23.60	23.67	23.87
M_0 N_{120} P_{30}	49.13	47.87	46.47	47.82	23.83	23.00	20.10	22.31
M_6 N_{80} P_0	55.70	54.92	54.20	54.94	26.97	25.67	25.17	25.93
Mean	52.31	51.70	50.57	51.52	24.63	23.59	22.53	23.58
LSD *(p<0.05)*	R=1.39, W=0.96, RxW=NS				R=1.62, W=0.89, RxW=NS			
15-30 cm					**90-120 cm**			
M_0 N_{120} P_0	30.00	28.50	28.20	28.90	21.10	22.67	22.17	21.98
M_6 N_{80} P_{30}	32.03	30.50	30.27	30.93	25.47	25.47	23.47	24.80
M_0 N_{120} P_{30}	28.77	27.87	34.07	30.23	22.13	22.33	21.07	21.84
M_6 N_{80} P_0	36.30	33.70	33.40	34.47	27.27	24.90	28.27	26.81
Mean	31.78	30.14	31.48	31.13	23.99	23.84	23.74	23.85
LSD *(p<0.05)*	R=2.04, W=NS, RxW=3.60				R=1.30, W=NS, RxW=NS			
30-60 cm					**120-150 cm**			
M_0 N_{120} P_0	26.67	26.47	24.50	25.88	24.43	22.60	24.43	23.82
M_6 N_{80} P_{30}	27.07	26.63	26.85	26.85	25.00	24.83	24.33	24.72
M_0 N_{120} P_{30}	24.63	21.90	20.60	22.38	22.73	22.17	30.87	25.26
M_6 N_{80} P_0	29.37	26.70	26.60	27.56	29.47	28.93	25.40	27.93
Mean	26.93	25.43	24.64	25.66	25.41	24.63	26.26	25.43
LSD *(p<0.05)*	R=1.30, W=0.83, RxW=1.65				R=2.33, W=NS, RxW=3.13			

Table.4 Depth wise distribution of total Cu (mg kg^{-1}) under rice-wheat system

Treatments of rice	Rates of P applied to wheat (kg P_2O_5 ha^{-1})			Mean	Rates of P applied to wheat (kg P_2O_5 ha^{-1})			Mean
	P_0	P_{30}	P_{60}		P_0	P_{30}	P_{60}	
0-15 cm					**60-90 cm**			
M_0 N_{120} P_0	7.70	7.64	7.43	7.59	9.60	9.48	9.57	9.55
M_6 N_{80} P_{30}	8.29	8.27	7.88	8.14	9.73	9.73	9.39	9.62
M_0 N_{120} P_{30}	7.63	7.44	7.20	7.42	8.97	9.23	9.01	9.07
M_6 N_{80} P_0	9.43	9.23	8.23	8.97	9.93	9.40	9.33	9.56
Mean	8.26	8.14	7.69	8.03	9.56	9.46	9.32	9.44
LSD *(p<0.05)*	R=0.17, W=0.15, RxW=0.29				R=0.36, W=NS, RxW=NS			
15-30 cm					**90-120 cm**			
M_0 N_{120} P_0	8.87	8.77	8.67	8.77	9.47	9.33	8.97	9.25
M_6 N_{80} P_{30}	10.32	9.77	9.47	9.85	9.57	9.87	9.17	9.54
M_0 N_{120} P_{30}	8.10	7.68	7.87	7.88	9.20	8.77	9.30	9.09
M_6 N_{80} P_0	9.67	9.74	9.77	9.72	9.60	9.36	9.30	9.42
Mean	9.24	8.99	8.94	9.05	9.46	9.33	9.18	9.32
LSD *(p<0.05)*	R=0.27, W=NS, RxW=NS				R=NS, W=NS, RxW=NS			
30-60 cm					**120-150 cm**			
M_0 N_{120} P_0	8.43	9.57	9.23	9.08	9.33	8.97	9.63	9.31
M_6 N_{80} P_{30}	9.53	9.30	9.37	9.40	9.73	9.40	9.10	9.41
M_0 N_{120} P_{30}	9.23	8.57	8.80	8.87	8.73	8.98	9.90	9.20
M_6 N_{80} P_0	9.87	9.77	9.50	9.71	9.63	9.67	9.93	9.74
Mean	9.27	9.30	9.22	9.26	9.36	9.25	9.64	9.41
LSD *(p<0.05)*	R=0.55, W=NS, RxW=NS				R=NS, W=NS, RxW=NS			

Table.5 Depth wise distribution of total Fe (%) under rice-wheat system

Treatments of rice	Rates of P applied to wheat (kg P_2O_5 ha^{-1})			Mean	Rates of P applied to wheat (kg P_2O_5 ha^{-1})			Mean
	P_0	P_{30}	P_{60}		P_0	P_{30}	P_{60}	
0-15 cm					**60-90 cm**			
M_0 N_{120} P_0	1.43	1.38	1.37	1.39	1.60	1.63	1.63	1.62
M_6 N_{80} P_{30}	1.47	1.45	1.43	1.45	1.64	1.64	1.67	1.65
M_0 N_{120} P_{30}	1.33	1.37	1.25	1.31	1.57	1.59	1.62	1.59
M_6 N_{80} P_0	1.51	1.49	1.48	1.49	1.67	1.69	1.73	1.70
Mean	1.43	1.42	1.38	1.41	1.62	1.64	1.66	1.64
LSD *($p<0.05$)*	R=NS, W=NS, RxW=NS				R=0.02, W=0.01, RxW=NS			
15-30 cm					**90-120 cm**			
M_0 N_{120} P_0	1.44	1.45	1.44	1.44	1.60	1.61	1.63	1.62
M_6 N_{80} P_{30}	1.45	1.46	1.49	1.47	1.66	1.65	1.67	1.66
M_0 N_{120} P_{30}	1.40	1.41	1.43	1.42	1.56	1.58	1.60	1.58
M_6 N_{80} P_0	1.50	1.53	1.55	1.53	1.69	1.72	1.74	1.71
Mean	1.45	1.46	1.48	1.46	1.63	1.64	1.66	1.64
LSD *($p<0.05$)*	R=0.02, W=0.02, RxW=NS				R=0.01, W=0.01, RxW=NS			
30-60 cm					**120-150 cm**			
M_0 N_{120} P_0	1.54	1.54	1.56	1.55	1.70	1.70	1.73	1.71
M_6 N_{80} P_{30}	1.57	1.58	1.62	1.59	1.74	1.74	1.76	1.75
M_0 N_{120} P_{30}	1.45	1.48	1.52	1.48	1.64	1.66	1.67	1.66
M_6 N_{80} P_0	1.63	1.63	1.65	1.64	1.78	1.79	1.81	1.79
Mean	1.55	1.56	1.59	1.56	1.72	1.72	1.74	1.72
LSD *($p<0.05$)*	R=0.02, W=0.01, RxW=NS				R=0.02, W=0.01, RxW=NS			

Table.6 Depth wise distribution of total Mn (mg kg^{-1}) under rice-wheat system

Treatments of rice	**Rates of P applied to wheat (kg P_2O_5 ha^{-1})**			**Mean**	**Rates of P applied to wheat (kg P_2O_5 ha^{-1})**			**Mean**
	P_0	P_{30}	P_{60}		P_0	P_{30}	P_{60}	
0-15 cm					**60-90 cm**			
M_0 N_{120} P_0	216.3	215.7	213.7	215.2	332.5	331.5	327.7	330.6
M_6 N_{80} P_{30}	217.2	215.7	217.1	216.7	337.0	335.8	334.3	335.7
M_0 N_{120} P_{30}	210.1	205.3	203.6	206.4	321.2	317.0	316.5	318.2
M_6 N_{80} P_0	219.8	218.8	218.7	219.1	345.3	343.3	340.4	343.0
Mean	215.8	213.9	213.3	214.3	334.0	331.9	329.7	331.8
LSD *($p<0.05$)*	R=0.95, W=0.92, RxW=1.84				R=1.30, W=1.03, RxW=NS			
15-30 cm					**90-120 cm**			
M_0 N_{120} P_0	290.4	293.2	295.5	293.0	349.1	346.5	342.8	346.1
M_6 N_{80} P_{30}	297.4	299.6	303.3	300.1	352.7	350.8	349.6	351.0
M_0 N_{120} P_{30}	285.5	289.2	288.0	287.6	340.3	338.3	332.7	337.1
M_6 N_{80} P_0	305.3	303.7	302.3	303.8	360.9	358.3	354.8	358.0
Mean	294.7	296.4	297.3	296.1	350.8	348.5	344.9	348.1
LSD *($p<0.05$)*	R=1.04, W=0.97, RxW=1.95				R=1.41, W=1.01, RxW=NS			
30-60 cm					**120-150 cm**			
M_0 N_{120} P_0	310.8	306.9	305.5	307.7	373.9	370.7	371.8	372.1
M_6 N_{80} P_{30}	317.1	316.5	313.7	315.8	382.3	379.9	376.5	379.6
M_0 N_{120} P_{30}	304.8	299.1	297.2	300.4	367.5	364.5	363.6	365.2
M_6 N_{80} P_0	326.4	324.3	321.3	323.9	386.5	384.5	384.6	385.2
Mean	314.8	311.7	309.3	311.9	377.6	374.9	374.1	375.5
LSD *($p<0.05$)*	R=1.50, W=1.59, RxW=NS				R=1.93, W=1.08, RxW=NS			

The interaction between rice and wheat treatments is observed as significant. Similar results were observed by Sharma *et al.*, (2000) and Lu *et al.,* (2004), who reported that the continuous use of rice-wheat cropping system year after year reduced the Mn due to leaching down to lower depth of soil.

Vitamin C content in different hoblies of different district of Karnataka is tabulated in table 2. Vitamin C content was significantly high in almost all the hoblies of Shimoga district. Banagi and Muturu hobli were having (1056.82 and 1056.81ppm) of vitamin C content and followed by kasaba hobli of Hosanagara taluk (1056.77ppm), Talaguppa hobli (1056.66ppm) and kasaba hobli of Bhadravathi taluk (1056.55ppm). Less concentration was identified in kasaba hobli of Shimoga taluk 352.11ppm, nidige hobli 352.33ppm respectively.

In Chikkamagalur district, more vitamin c content was found in Kasaba hobli of Sringeri taluk (1056.84ppm), Kalasa and Kasaba hobli of Mudigere taluk had 1056.53 and 1056.51ppm of vitamin C content and less concentration was estimated in Lakya and khandya hobli of Chikkamagalur taluk (528.22 and 528.68ppm), and Kasaba hobli of Narasimharajapura taluk (528.67ppm).

There was no significant difference of vitamin C content in Davangere district. The vitamin c ion concentration was similar in Santhebennuru and Anagodu hobli (1056.33ppm). In Kasaba hobli of Honnali taluk and in Belaguthi hobli vitamin C content was found to be (1056.50 and 1056.74ppm).

In Chitradurga district there was a significant variation of vitamin C content. The highest concentration of vitamin C was found in Kasaba hobli of Holalkere taluk (792.63ppm) and less content was observed in Ramagiri hobli (264.56ppm) and followed by Imangala hobli, Hireguntanuru hobli (264.75ppm and 264.79ppm).

In Kadaba hobli of Dakshina kannada district were having more concentration of vitamin C content (792.79ppm), and followed by Bantwala and vitla hobli (792.52 and 792.50ppm) and Mangaluru B and Mudabidre were having less content of vitamin C (264.75 and 264.56ppm). In Udupi district high concentration of vitamin C was identified in Kota hobli (792.22ppm) and less content was found in Vandse hobli 264.37ppm and followed by Ajekaru and Karkalla hobli of karakala taluk (264.55ppm and 264.56ppm).

Vitamin B_6, also called pyridoxine, is a water-soluble nutrient that is part of the B vitamin family. B vitamins, including vitamin B6, help support adrenal function, help calm and maintain a healthy nervous system, and are necessary for key metabolic processes.

Vitamin B6 acts as a coenzyme in the breakdown and utilisation of carbohydrates, fats and proteins. Vitamin B_6 helps in the production of neurotransmitters, the chemicals that allow brain and nerve cells to communicate with one another, ensuring that metabolic processes such as fat and protein metabolism run smoothly, and is important for the functioning of immune system in older individuals.

The result of samples analysed for vitamin B6 from 117 locations of six important areca growing districts of Karnataka are presented in the table 1. In general, the contents of vitamin B6 ranged from 10 to 91 ppm. Higher vitamin B6 contents were in Anwatti (52.33), Kigga (72.67), Bilichodu (84.67), Turuvanooru (72.67), Uppinangadi (91.22) and Ajekaru (81.00) of Shimoga, Chikkamagaluru, Davanagere, Chitradurga, Dakshina kannada and Udupi districts respectively. It is interesting to notice that, in about 30 percent of locations the content of vitamin B6 was only 10 ppm. Talukwise variation was highly significant with a range of 10.24 to 88.38 ppm. Highest vitamin B6 was observed in samples of Puttur (88.38) followed by Karkala (67.98) and Hosadurga (56.44) whereas lowest concentration was in Hosanagara (10.21). The mean vitamin content was lowest in Shimoga district (15.68) and highest was in Dakshina Kannada district (50.49).

Regarding vitamin C contents, there were a significant differences among the hoblies of all the six districts of Karnataka. Vitamin content in different hoblies has been presented in table 3. The samples of 8 hoblies *viz.* Alduru, Panchanahalli, Gonibidu, Balehonnuru, Basavapatna, Kasaba, Arsikere and Govinkovi contained highest vitamin C content of 1320 ppm but lower content was found in 10 hoblies viz. HireguntImangala, Kasaba, Ramagiri, Mangaluru, Mudabidre, Ajekaru, Karkala, Kundapura and Vadse wherein its concentration was only 264 ppm. Highly significant variations existed in vitamin C content among all the 35 taluks of 6 districts (Table 4). Mean over all the taluks indicated that highest vitamin C content (1012 ppm) was in Davanagere district followed by Shimoga and Chikamagaluru districts (897 ppm). Similarly, Areca nut Research and Development Foundation (ARDF 2015), Sirsi have reported that, vitamin-A and vitamin B-6 were found in traces in areca nut samples of Karnataka.

The determination of vitamin B6, it should be noted that vitamin B6 are usually unstable and therefore the reference and sample protocols must be handled with great care while using HPLC. Separation and quantification should be done with a high level of precision and suitable methods. HPLC method for analysing vitamin B6 is common but a single run HPLC method for simultaneous analysis of vitamins are undocumented. It is therefore important to have a single most sensitive HPLC method, which is robust, rapid and efficient for determining all the water-soluble vitamins in a single run. Both hobli wise and taluk wise areca samples recorded highly significant variations in vitamin B6 contents with a range of 91.33 to 10.00 ppm.

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