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The Effect of Urea on the Concentration of Fe, Mn, Zn and Cu in Rice Plant at Two Different Soils

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ABSTRACT

This research aims to investigate the effect of urea level on concentration of Fe, Mn, Zn and Cu in shoot and root of the Hashemi rice (*Oryza sativa* L.) in two soils with clay loam and loamy sand texture. This investigation is factorial experiment in a completely randomized design with four replicates and include urea in three levels (0, 100, and 200 mg N/kg), two types of soil and in greenhouse condition. Results of the study show the type of soil has a meaningful effect on concentration of Fe and Mn in root and shoot, Cu on shoot and Zn on root of the rice. The high concentration of Zn and Mn in shoot, and Fe and Mn in root is shown in loamy sand soil and high concentration of Fe in shoot and Cu in root is represented in clay loam soils. Interaction of type of soil and urea show that concentration of Mn in root decreases in 100 mg N/kg on clay loam soil, while the concentration of Cu in root is increased. Concentration of Cu in shoot decreases in 100 mg N/kg on loamy sand soils, while using urea and increasing its level in clay loam soil increases its concentration. Concentration of Zn in root is also meaningfully increased using 100 mg N/kg in loamy sand soils.

KEYWORDS: Fe, Urea, Rice, Zn, Cu, Mn, Soil Type.

1. INTRODUCTION

Plant cultivation, type of soil, and type and amount of fertilizer can change pH and nutrient availability [1-4]. Nitrogen play important role in cation-anion balances since it's the most nutrient absorbed by most of the plants [2]. Using urea has effect in nutrient availability in soil solution by influencing soil pH. Results represented that long term using of urea increases concentration of Zn, P, Fe and Mn of soil by decreasing pH. On the other hand, decreasing pH of soil will decrease CEC and therefore decreases holding capacity of soil for cations such as Ca, Mn and K [5, 6]. In their greenhouse study, Hu-Lin *et al.* [7] reported that using urea (0, 80, 160 and 320 kg N/h) a increases concentration of Fe, Mn, Cu and Zn in shoot of two genotypes of rice and the most increase was reported at kg N/ha 160 level. Rasouli and Maftoun [8] added 0, 75 and 150 mg/kg of urea to soil and reported that it increases concentration of Fe, Mn, Cu and Zn in shoot of rice. Results of Hosseiny and Maftoun [9] shows using 400 mg N/kg urea increases concentration of Zn in shoot of rice compared with other levels (0, 50 and 100 mg N/kg). Using 150 mg N/kg of urea in three texture of calcareous soil (clay loam, loamy sand, and sandy), Boostani and Ronaghi [10] reported that concentration of Fe, Mn and Cu in shoot of popcorn is increased. Jana *et al.* [11] represented adding nitrogen chemical fertilizer increases concentration of Mn in rice. This result is in accordance with findings of Cheng and Jie [12] and Yuan *et al.* [13].

Fe, Zn, Cu and Mn are essential micronutrients for plant growth. One of the main problems in recent years is lack of these elements in different areas of the world, especially in calcareous soils [14]. Using nitrogen fertilizers increases the vegetative period and causes lack of Fe, Zn, Cu and Mn in soil [15]. Shafea and Saffari [16] reported that using urea increases Fe at the shoot of popcorn but decreases Cu and Zn. Brohi *et al.* [17] studied the effect of five levels of urea (0, 60, 120, 180 and 240 kg N/ha) on concentration of micronutrients in shoot of the rice in clay loam soil and reported that increasing urea decreases concentration of Fe, Mn and Zn. They believe this is due to decrease of concentration of elements due to dilution. Results of the study of Rahman *et al.* [18] represents that using 100 kg N/ha of urea in calcareous soil decreases transfer and absorption of Zn to shoot of the rice, which is due to disturbance of $HCO3^-$ ions in plant. Adiloglu [19] uses ammonium fertilizer from ammonium nitrate source in three levels (0, 50 and 100 kg N/ha) on calcareous soil and shows concentration of Fe, Mn and Cu is decreased in popcorn plant. Kalbasi *et al.* [20] believe in calcareous soils, Fe, Mn and Cu can't be sufficiently absorbed by the plant. Therefore, sedimentation of these elements in soil causes deficiency of plant.

Limitation of oxygen in submergence soils decreases transfer of ammonium obtained by hydrolyzing of urea to nitrate. However, mineral nitrogen in wetland rice soils is mostly observed as ammonium nitrogen [21]. The amount of cation and anion absorption in dissolved nutrient [2] and microbial activity (nitration and removal of nitration) has effect on pH. Preferential uptake of ammonium in neutral and alkaline soils and decrease of

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rhizosphere pH increases availability of micronutrients [22]. Hence, in this research the effect of urea in availability of micronutrients of soils for rice plant in loamy sand and clay loam soils is studied.

2. MATERIALS AND METHODS

In this research, two types of soil with loamy sand and clay loam texture were selected. Loamy sand soil was taken from Khalat Poushan research station of agricultural faculty of Tabriz University, and clay loam soil was taken from a land around Spiran village of Tabriz. Soil samples were taken from 0-20 cm depth and after drying on air and passing 2 mm sieve, physical [23] and chemical characteristics [24, 25] of soil were measured, the results of which are represented in Tables 1. 3 kg soil was added to pots. This investigation is factorial experiment in a completely randomized design with four replicates and include urea in three levels (0, 100 and 200 mg N/kg), and two types of soil. Urea was added in three times (before planting, tillering and stem elongation) and chemical fertilizers were added based on results of soil test and recommended fertilization of rice plant [26] in the form of solution and before planting. One percent of dry manure was also added to all samples. Soil of the pots was kept for two weeks in submergence state with a little water (about one inch) on soil surface to reach relative balance. About 10 germinated Hashemi rice seeds (*Oryza sativa L.*) were planted and after two weeks the amount of plants in each pot changed to 4. After placement of the rice plant, water elevation was raised to five inches and kept in this level by daily adding water to it up to the end of growing period. The test was carried on for three months in greenhouse condition. At the end of growth period, the shoot of plant was cut from crown and their weight was measured using digital scale. Then, shoot and root slices were placed in paper bags and transferred to oven and dried for three days in 75 C. After that, samples were taken out of oven and their dry weight was measured by g±0.001 scale. After drying, samples were crashed and passed 0.5 mm sieve to create a uniform sample. Concentration of Fe, Mn, Zn and Cu of the obtained extract was measured by atomic absorption model tool made in Shimadzo Co. Japan, Model AA-6200.

To study the effect of urea and moisture level of soil on dissolved pH of soil, first a polyethylene hose was placed on bottom of the pots, in a way that one end of it was in the pot and the other end was out of the pot. To extract clear extraction of each pot, the hoses were covered with a layer of glass wool. Control and experimental treatments were 200 mg N / kg. The texture of under study soil was loamy sand and clay loam. Required dissolved chemical fertilizers were mixed with the soil. Dissolved urea was injected to pots three times with 50 ml needle. The solution sediment at the bottom of the pots was extracted with 50 ml needle, placed in a polyethylene tube and its pH was immediately measured. After each measurement of pH, the solution was again returned to the pots to prevent any changes in chemical composition caused by removal of water lotion. Statistical analysis of data was done using MSTATC software and means were compared using LSD test with probability level of 5%.

Table 1a. Some chemical and physical properties the soils used in study.

Texture	Sand (%)	Silt (%)	Clay (%)	$CaCO_3$ (%)	O.C (%)	SP	FC (%)	pH (1:1)	EC (1:1) (dS/m)
Loamy sand	70	18	12	0	0.11	30	15	7.63	0.11
Clay loam	39	38.5	22.5	15.25	0.58	39	19	7	0.47

Table 1b. Concentration of absorbable elements of soil

Texture	P (mg/kg)	K (mg/kg)	Na (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	N (%)	Cu (mg/kg)
Loamy sand	5.7	250	108.8	1.8	1.1	0.85	0.08	1.3
Clay loam	8.7	556.4	325.7	3.98	7.01	0.52	0.02	2.2

3. RESULTS AND DISCUSSION

Concentration of Mn in shoot of the rice:

Variance analysis shows the main effect of soil in probability level of 1% is significant in concentration of Mn in shoot, but the main effect of fertilizer and their interaction is not significant (Table 2). Comparing means show that concentration of Mn in shoot in loamy sand soil is significantly more than clay loam soil (Table 3). Interaction of type of soil and urea shows the concentration of Mn of shoot in loamy sand soil in each level of fertilizer is more than clay loam soil, while the influence of urea on concentration of Mn in shoots of both types of soil is not meaningful (Figure 3). Optimal concentration of Mn in texture of rice plant is 40-700 mg Mn/kg dw [27]. Concentration of Mn in shoot in loamy sand soil is more than optimum while in clay loam is optimum. This is due to the use of chemical fertilizer on sulfate Mn in loamy sand soil. Results of measuring dissolved pH of both types of the soils show that after submergence, dissolved pH of the soil decreases (Figures 1 and 2). Reduction of pH increases solubility of Mn and effects its concentration and absorption. On the other hand, submergence of soil increases concentration of Fe and Mn ions and creates a struggle between them for absorption

[28]. In the cases where Fe is higher than normal, solubility and migration of plant is limited and its amount decreases. Concentration of Fe in shoo is more in clay loam soil and less in loamy sand soil (Table 3). Therefore, due to the struggle between Fe and Mn, absorption of Mn in loamy sand soil is more. Mehdi and DeDatta [29] reported changes of Mn solution of soil are like Fe but in submergence, the increase of dissolved Mn in soil is more than Fe. Results of Adiloglu [19] shows the concentration of Mn in shoot of popcorn in loamy sand soil is more than clay loam. Studying the effect of submergence on availability and absorption of micronutrient in loamy sand and sand soils, Bjerr and Schierup [30] reported due to high availability of Mn in sand soil (CEC and low pH) compared to loamy sand soil, concentration of Mn is more in aerial part of atmosphere.

Table 2. Variance analysis of the effect of soil type and urea level on concentration of micronutrients in shoot of the rice

S.O.V	df	Mean Squares			
		Mn	**Fe**	**Zn**	**Cu**
Block	3	13863.28$^{n.s}$	955.06$^{n.s}$	45.61$^{n.s}$	0.24$^{n.s}$
Soil	1	1144246**	6661.34*	1812.9**	8.46$^{n.s}$
urea	2	1382.97$^{n.s}$	669.79$^{n.s}$	58.12$^{n.s}$	99.37**
Soil × urea	2	2601.22$^{n.s}$	817.51$^{n.s}$	15.15$^{n.s}$	16.7**
Error	15	6137.47	1340.38	44.5	2.19
CV (%)		15.8	28.07	9.97	7.99

* , ** means significant in 0.01 and 0.05 level of probability respectively and ns: Non significant

Table 3. Comparing mean concentration (mg/kg dw) of micronutrients on shoot of the rice under effect of soil type and urea level

Treatments	Levels	Mn	Fe	Zn	Cu
Soil	Loamy sand	714.3a	113.8 b	75.6a	17.9a
	Clay loam	277.6b	147.1a	58.2b	19.1a
Urea (mg N/kg)	control	485.2a	136.6a	65.0a	16.3 b
	100	510.6a	134.8a	65.7a	16.7b
	200	491.9a	119.9a	69.9a	22.6a

Means followed by the same letter in each column is not significantly different according to LSD s test (P<0.05).

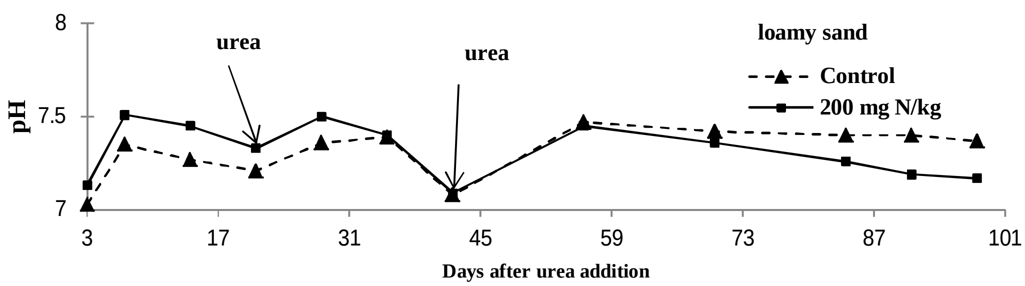

Figure 1. Effect of urea levels on pH changes of loamy sand in rice pots

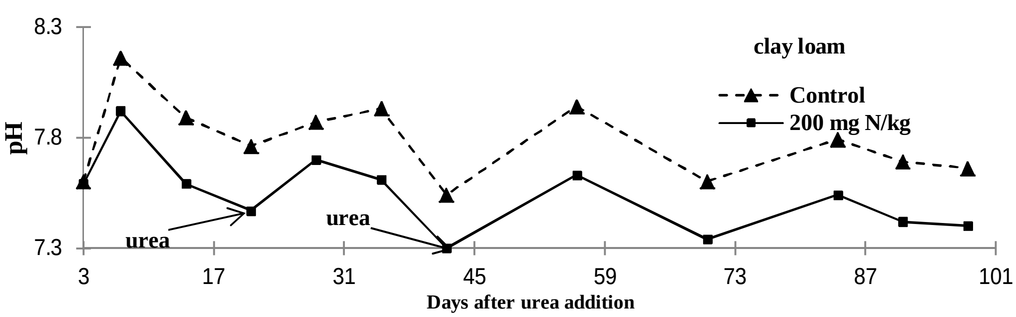

Figure 2. Effect of urea levels on pH changes of clay loam in rice pots

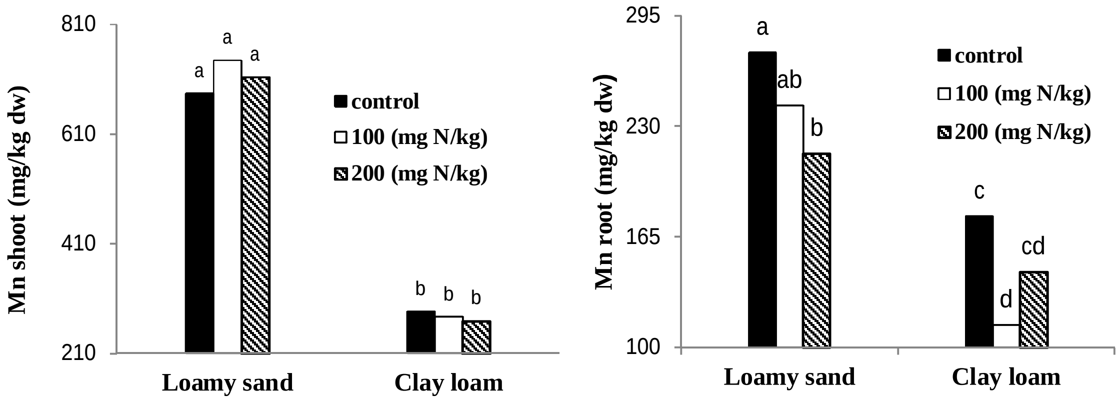

Figure 3. Interaction effect of urea levels and soil type on concentration of Mn in shoot and root of rice

Concentration of Mn in rice root:

Variance analysis shows the main effect of soil and fertilizer in probability level of 1% and their interaction is significant in 5% probability level on concentration of Mn in root of the rice (Table 4). Comparing means show that concentration of Mn in root in loamy sand soil is significantly more than clay loam soil. Concentration of Mn in root of the sample was statistically more than other two levels of concentration. Using urea decreases concentration of Mn in root (Table 5). Increasing concentration of Mn in loamy sand soil can be due to high availability of Mn. Reducing concentration of absorbable Mn in clay loam soil can be due to calcareous soil and formation of Mn carbon sediments in soil [20]. Interaction of type of soil and urea shows the concentration of Mn of root in 200mg N/kg in loamy sand soil or 100 mg N/kg in clay loam soil decreases significantly regarding the sample group (Figure 3). Using urea increases growth of plant shoot (Figure 4), therefore, increases transfer rate of this element from root to shoot [7, 17]. As a result, concentration of Mn decreases in root. Brohi *et al.* [17] also reported similar findings. Due to the reduction of oxygen in submergence state, hydrolyzing urea is stopped in nitration level and extra amount of ammonium ions, struggle between Mn and Al for absorption by plant is increased. Also the amount of transferrable and dissolved Mn can be decreased due to high fix ammonium in transferrable places of clay loam soil.

Table 4. Variance analysis of the effect of soil type and urea level on concentration of micronutrients in root of the rice

S.O.V	df	Mean Squares			
		Mn	**Fe**	**Zn**	**Cu**
block	3	$794.94^{n.s}$	$^{n.s}29954.88$	$424.83^{n.s}$	$76.23^{n.s}$
soil	1	57979.31^{**}	$^{*}56852.4$	$909.71^{n.s}$	1511.94^{**}
urea	2	5840.57^{**}	$^{n.s}14069.84$	$2037.95^{n.s}$	689.56^{**}
Soil × urea	2	1784.43^{*}	$^{n.s}872.06$	$2491.54^{n.s}$	1192.07^{**}
Error	15	478.27	11152.02	6360.27	95.51
CV (%)		11.27	14.48	14.76	14.59

* , ** means significant in 0.01 and 0.05 level of probability respectively and ns: Non significant

Table 5. Comparing mean concentration (mg/kg dw) of micronutrients on root of the rice under effect of soil type and urea level

Treatments	Levels	Mn	Fe	Zn	Cu
soil	Loamy sand	243.3a	776.2a	145.6a	59.1 b
	Clay loam	144.9 b	680.8b	133.3a	74.9 a
Urea (mg N/kg)	control	225.3a	776.8 a	132.3a	64.5b
	100	177.7b	714.8 a	152.5a	77.3a
	200	179.4b	696.9a	133.6 a	59.2 b

Means followed by the same letter in each column is not significantly different according to LSD s test ($P<0.05$).

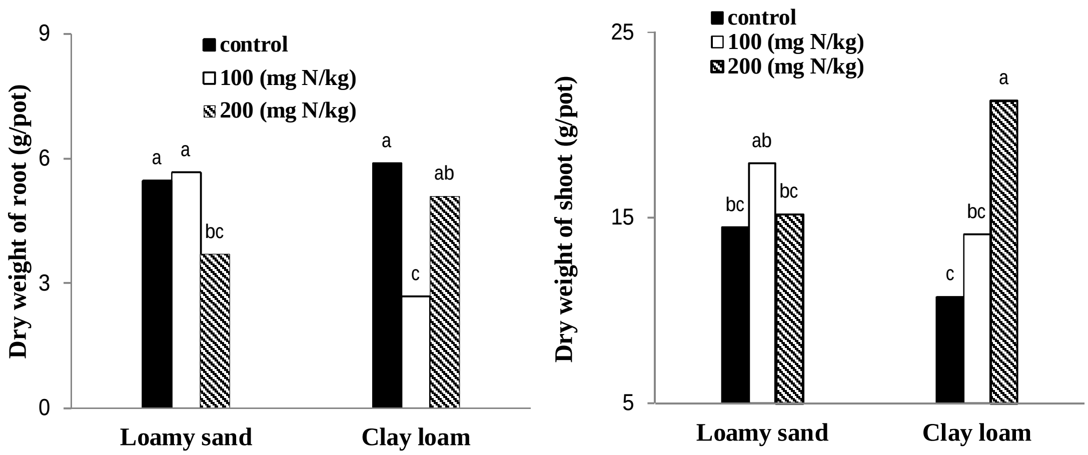

Figure 4. Interaction effect of urea levels and soil type on dry weight of shoot and root

Concentration of Fe in shoot of rice:

Variance analysis shows the main effect of soil in probability level of 5% is significant in concentration of Fe in shoot of the rice, while main effect of fertilizer and their interaction is not meaningful (Table 2). Comparing means show that concentration of Fe in shoot in clay loam soil is meaningfully more than loamy sand soil. There is no significant effect between levels of fertilizer regarding concentration of Fe in shoot (Table 3). Increasing concentration of Fe in clay loam soil can be due to high concentration of absorbable Fe compared to loamy sand soil. Due to struggle between Fe and Mn, concentration of Mn in shoot in clay loam soil is less than loamy sand. By absorbing more Mn, solubility and migration of Fe becomes limited and therefore decreased. Another factor can be accumulation of Fe in root, which can't be transferred to shoot due to less motivation of Fe. Optimal range of concentration of Fe in texture of rice plant is 75-150 mg/kg dw, and concentration higher than 300 mg/kg dw is poisonous [27]. Concentration of Fe in shoot of both loamy sand and clay loam soil is optimal.

Concentration of Fe in root of rice*:*

Variance analysis shows the main effect of soil in probability level of 5% is significant in concentration of Fe in root of the rice, while main effect of fertilizer and their interaction is not significant (Table 4). Comparing means show that concentration of Fe in root in loamy sand soil is significantly more than clay loam soil. There is no significant effect between levels of fertilizer regarding concentration of Fe in root (Table 5). The volume of root in loamy sand soil with light texture is more than clay loam soil; therefore, availability of nutrients in this soil is faster than clay loam soil. Also, in clay loam soil, concentration of Fe in root decreases due to transfer of absorbed Fe to shoot. Coarse, scattered and damaged root with dark brown cover on root are poisoning effects of Fe, which causes death of most of the roots [27]. It seems this is due to sedimentation of Fe in the root due to release of oxygen by root and oxidation of Fe and its change to Fe oxides. Reddish brown is normal in these cases and shows healthy roots, but over increase of Fe can damage roots and prevent root growth.

Concentration of Zn in shoot of rice:

Variance analysis shows the main effect of soil in probability level of 1% is significant in concentration of Zn in shoot of the rice, while main effect of fertilizer and their interaction is not significant l (Table 3). Comparing means show that concentration of Zn in shoot in loamy sand soil is meaningfully more than clay loam soil. Three fertilizer levels have no meaningful differences regarding concentration of Zn in shoot (Table 4). Interaction between type of soil and urea show that concentration of Zn in shoot in both soils has not changed significantly after using urea (Figure 5). Some researchers believe than reducing concentration of Zn due to nitrogen fertilizer is not significant in plants. They believe the meaninglessness of Zn reduction is due to high or sufficient amount of Zn in soil and its availability to plant [31-35]. Increase of Zn in shoot in loamy sand soil can be due to its high transfer velocity from root to shoot. Zn sediments as ZnSin sodium and calcareous soils, and is adsorbed by CaCO3 or Fe oxides. Inanaerobic conditions, insoluble zincphosphate forms [27]. Due to calcareous clay loam soil, mentioned causes can be regarded as the causes of low Zn in clay loam compared to loamy sand soil. Optimal range of concentration of Zn in texture of rice plant is 25-50 mg Zn/kg dw and more than 500 mg Zn/kg dwis poisonous [27]. In both soils, concentration of Zn in shoot of the rice plant is a little more than optimal. This is due to the use of Zn sulfate fertilizer in both soils.

Concentration of Zn in root of rice:

Variance analysis shows the main effect of soil and fertilizer and their interaction in root is not meaningful (Table 4). Comparing means show that concentration of Zn in root in loamy sand soil is not meaningfully different from clay loam soil. There no meaningful differences between fertilizer levels regarding concentration of Zn in root (Table 5). Interaction between type of soil and urea show that concentration of Zn in root in 100 mg N/kg level of loamy sand soil is more than control level. Increasing concentration of Zn in root by urea can be related to reduction of concentration of Mn in root of the plant (Figure 3). Due to antagonist effects of Zn and Mn and reducing concentration of this element in root by using urea, concentration of Zn in root is increased (Figure 5). The most dry weight of root is in 100 mg N/kg level. Reducing dissolved pH of soil can cause increase of concentration of dissolved Zn and its absorption in root.

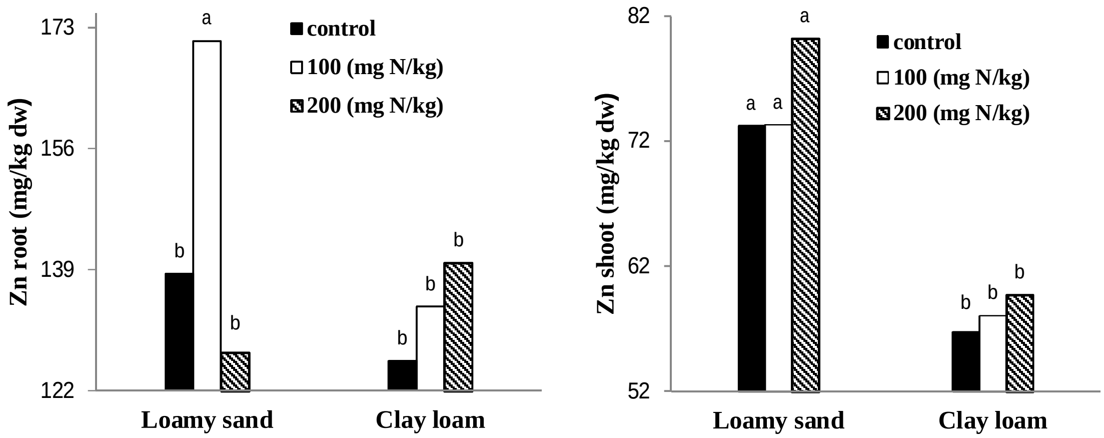

Figure 5. Interaction effect of urea levels and soil type on concentration of Zn in shoot and root of rice

Concentration of Cu in shoot of the rice:

Variance analysis shows the main effect of soil in probability level of 1% is not meaningful, while main effect of fertilizer and their interaction is meaningful (Table 2). Comparing means show that concentration of Cu in shoot in clay loam soil is meaningfully more than loamy sand soil. Statistically, concentration of Cu in shoot in 200 mg N/kg level is more than two other levels. Using urea increases concentration of Cu in shoot. Concentration of Cu in shoot is higher in 200 mg N/kg level (Table 3). Hu-lin *et al*. [7], and Rasouli and Maftoun [8] reported similar findings. Using urea in clay loam soil meaningfully increases concentration of Cu in shoot (Figure 6). There is a positive relation between urea and dry weight of shoot of rice, and using urea increases absorption and transfer of micronutrients from root to shoot (Figure 4). Hu-lin *et al.* [7] reported similar findings. By decreasing pH of the soil, urea increases the solubility and mobility of Cu in soil which leads to its absorption by plant. Lorenze *et al*. [36] represented the abundance of ammonium and potassium ions increases micronutrient ions in soil solution and its absorption in plant. Using urea and loamy sand soil meaningfully decreases concentration of Cu in shoot in 100 mg N/kg level compared to other levels (Figure 6). This is due to increase in growth of shoot and decreasing transfer rate of Cu to shoot. These results indicate nitrogen fertilizer increases absorption and transfer of micronutrients from root to shoot of the rice. Adiloglu [19] reported similar findings.

Concentration of Cu in root of the rice:

Variance analysis shows the main effect of soil, fertilizer and their interaction in probability level of 1% of Cu concentration in root is meaningful (Table 4). Comparing means show that concentration of Cu in root in clay loam soil is meaningfully more than loamy sand soil. Statistically, concentration of Cu in root is in 100 mg N/kg level is higher than two other level of fertilizer (Table 5). Pirdashty *et al.* [37] reported by using 100 kg/ha of chemical fertilizer from urea, sulfate potassium and super phosphate triple sources, the increase of Nitrogen, phosphor, Cu and Zn in root of bean is meaningful compared to non-fertilized control, but the increase of sodium, potassium, Fe and Mn of the root is not meaningful. In loamy sand soil, urea has no effect on Cu of the root but using 100 mg N/kg in clay loam soil meaningfully increases concentration of Cu in root compared to two other levels (Figure 6). This is due to reduction in concentration of Mn in clay loam soil and 100 mg N/kg level (Figure 3).

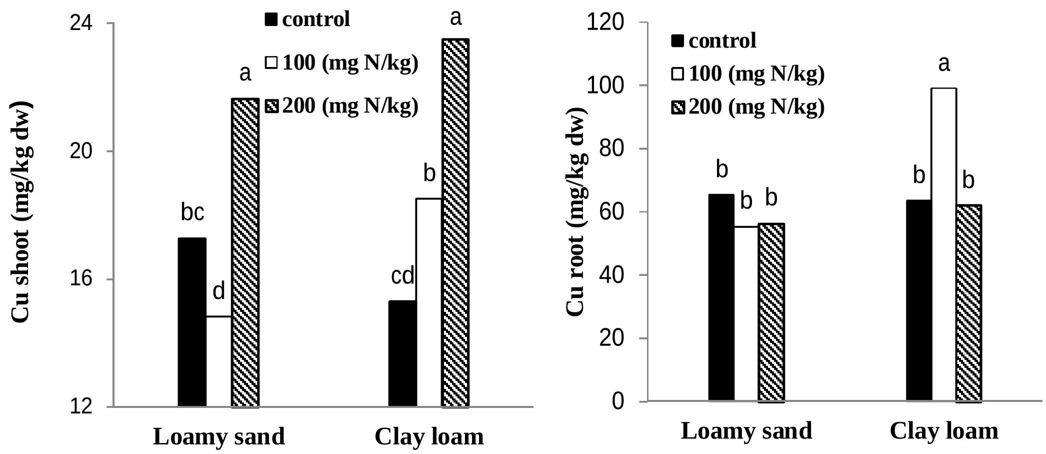

Figure 6. Interaction effect of urea levels and soil type on concentration of Cu in shoot and root of rice

4. CONCLUSION

Results of the study show the effect of type of soil in concentration of Fe, Zn and Mn of shoot, Fe, Mn, and Cu of the root is meaningful. The high concentration of Zn and Mn of shoot and Fe and Mn of root is observed in loamy sand soil and the high concentration of Fe in shoot and Cu in root is shown in clay loam soil. Interaction of type of soil and urea show the concentration of Mn in root of clay loam soil is decreased in 100 mg N/kg while concentration of Cu in root is increased, concentration of Cu in shoot in loamy sand soil is decreased in 100 mg N/kg while by using urea and increasing its level in clay loam soil, its concentration is increased. Concentration of Cu in root is also meaningfully increased when using 100 mg N/kg of it in loamy sand soil. Since using and increasing of urea increases dry weight of rice shoot in pots, the lean effect and the effect of urea on concentration of micronutrients is not meaningful. On the other hand, chemical fertilizers were used in pot soil. Solution of these fertilizers in submergence state increases availability of concentration of micronutrient elements in soil, therefore, concentration of these elements in shoot is optimal. To accurate investigation of the effect of urea, this study should be carried on in the same circumstances in farm and for nutrition of other plants.

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