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Research Article

The Effect of Urea and Level of Soil Moisture on Availability of Zinc and Copper in Two Different Soils in Vitro

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Abstract: The objective of this study is to find out the effect of urea and level of soil moisture (submergence and un-submergence) on availability of Zn and Cu nutrients in both loamy sand and clay loam soils in vitro. This investigation is factorial experiment in a Completely Randomized design (CRD) with two replications. Factors include moisture condition of soil at two levels (submergence and un-submergence), the amount of urea in three levels (0, 200 and 400 mg N/kg soil), two types of soil (loamy sand and clay loam)and time in four levels (1, 5, 15 and 45 days). Results show that concentration of extractable Cu and Zn in submergence is more than un-submergence. Adding urea increases concentration of dissolved Cu. When using both levels of urea in both submergence and un-submergence state, extractable Zn in loamy sand soil first decreases and then shows an increase. In clay loam soil, after using 200 mg N/kg, the amount of extractable Zn first decreases and then increases. Changes of extractable copper in both soils in three levels of fertilizer and un-submergence state are not meaningful, while using urea in submergence state increases extractable and dissolved copper in both soils.

Keywords: Copper, soil water, urea, zinc

INTRODUCTION

Fe, Mn, Zn and Cu are heavy metals that are essential in small amount for plant growth, but if their absorbable amount become high in soil, plants can be poisoned (Havlin *et al*., 2006). pH, soil moisture and the amount of Nitrogen fertilizer are effective factors on availability of these metals (Marschner, 2003). After using in the soil, urea faces various changes which effect pH and availability of nutrients. First, hydrolyzing of urea increases the amount of pH while this amount decreases by alteration of ammonium to nitrate (Gaudin and Dupuy, 1999). Long term using of urea, replacement of ammonium with alkali cations and production of hydrogen ion during nitration process, decreases the amount of pH (Juo *et al*., 1996). Mosadeghi *et al.* (2009) observed that using ammonium sulfate, urea and ammonium nitrate fertilizers increases total concentration of nitrite and nitrate in all treatments after 60 days and decreases concentration of dissolved ammonium. Nitration process in soil increases the total of nitrite and nitrate by passing of time. Gradual decrease in concentration of ammonium by passing of time is not only due to nitration process but ammonium adsorption by soil particles. Four days after adding urea, Asing *et al.* (2008) observed pH of soils increases from 6.8 to 5.5 and then decreases to 4.7. Initial increase of pH is due to hydrolyzing of urea and producing ammonium carbonate and second decrease is because of producing nitrate during nitration process. Fageria *et al.* (2010) observed that pH of the soil in 400 mg N/kg treatment of urea fertilizer increases from 6.6 to 6.2, compared to non-fertilized urea. Results of Mitchell *et al.* (1999) show the amount of dissolved zinc increases with the increase of urea. Mehdi and Dedatta (1997) reported using urea decreases dissolved zinc. He *et al.* (1999) reported that increase of ammonium meaningfully decreases pH of the soil around roots of grip fruit, concentration of zinc and Mn. Initial pH of this soil was 7.5. Philip *et al*. (1997) represented the long term using of Nitrogen fertilizer from two sources of urea and mono-ammonium phosphate, decreases copper but increases zinc. Haynes and Swift (1987) studied the effect of Zn and Cu increased by EDTA.

Another factor affecting availability of Zn and Cu on soil is moisture of the soil. Towfighy and Najafi (2002) show that after submerging paddy soils in north of Iran, extractable Zn decreases with DTPA. Studies show decreasing extractable Zn with DTPA in submergence state is meaningfully more thanun-submergence state (Sajwan and Lindsay, 1986; Towfighi and Najafi, 2002; Saleh *et al*., 2012, Valizadehfard *et al*., 2012). They believe this decrease is due to decrease of Redox parameter, increasing solubility of Fe, Mn, phosphorous and their anti-effect on availability of zinc. Saha *et al*. (1992) reported passing of time and submergence of soil decrease

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usable Zn which is due to sedimentation of Zn as different composites such as carbonate, phosphate or sulfide copper. Kashem and Singh (2001) show the concentration of dissolved Ni, Cd and Zn of soil decreases as the time of submergence increase. Van Laer *et al*. (2010) studied the effect of submergence on dissolved Zn of 12 acid Spodosls soil and reported after submergence, concentration of dissolved Zn decreases in one soil but increases in other 11 soils. The results show the effect of submergence on absorbable Zn of soil can be diverse depending on the type of soil. Patrick and Reddy (1978) stated copper chemistry in submergence soils are similar to Zn. Submergence soil changes active forms of Cu such as solution, transferrable and organic complex to inactive form; hence, availability of copper decreases after submergence (Weil and Holah, 1989; Saha and Mandal, 1998; Towfighi and Najafi, 2002; Saleh *et al*., 2012). Singh *et al*. (1982) reported extractable Cu first increases with DTPA of a calcareous soil with loamy sand texture in submergence and un-submergence state and then decreases. Its initial increase insubmergence state is due to decrease of pH in this soil. As mentioned above, according to Valizadeh *et al.* (2012) the aim of this research is to study the effect of urea and moisture level of soil on availability of Zn and Cu in two different types of soil in vitro.

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 KhalatPoushan 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 (Dane and Topp, 2002) and chemical characteristics (Richards, 1969;Page *et al*., 1982) of soil was measured (Table 1). This investigation is factorial experiment in a completely randomized design with two replicates. Treatments include moisture level of soils in two levels (submergence and un-submergence with 50% of saturated moisture), urea in three levels (0, 200 and 400 mg N/kg soil), two type of soil (loamy sand and clay loam) and time in four levels (1, 5, 15 and 45 day) (Singh and Yadav, 1981;Belliturk and Saglam, 2005). 10 g of dissolved urea fertilizer was added in mentioned three levels to each dry soil and placed into 50 ml centrifuge tubes and two levels of moisture (submergence and un-submergence) were generated in them by balancing. In submergence treatments, 3 cm of water were kept in soil surface. In un-submergence treatments, moisture of the soil was 50% of saturated moisture. Treatments were kept in 25±2 C for 45 days. Moisture of soil was balanced by daily distribution and adding distilled water. Since 3 cm of distilled water were used at the surface of soils for submerging, there should be enough DTPA to form K-lit with four elements of Fe, Mn, Zn and Cu to avoid dilution of extractor liquid during extraction in submergence state. These four elements have no struggle to bond with DTPA. In stated times, before extraction with DTPA, submergence treatments were centrifuged for 5 min with 3000 rpm. Then, the surface solution was transferred to polyethylene containers and all treatments were extracted in 1, 5, 15 and 45 day. Therefore, 20 ml of extractor was added to submergence and un-submergence treatments and was shaken for 15 min with velocity of 180 rpm. After that, they were filtered by What manfilter paper no. 42 inside polyethylene containers of 50 mL and extractions were kept in refrigerator in temperature of less than 3 C. Concentration of Zn and Cu of the obtained extract was measured by atomic absorption model tool made in Shimadzo Co. Japan, Model AA-6200.

The composition of the extractors was as 1M NH_4HCO_3+0.005M DTPA, pH = 7.6 (Soltanpur and Schwab, 1977) which is a multi-element extractor, that is, it can measure concentration of NO_3^-, P, K, Fe, Mn, Zn and Cu of the extraction, simultaneously. This is a low expense method since one extraction can determine the concentration of mentioned elements.

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 soils and about 3 kg of soil was placed in each pots. Dissolved urea was injected to pots three times with 50 mL needle. In half of the pots, 3-5 cm of water was kept at the surface of pots and the moisture of soil was maintained in suction of 0.3. Moisture of soil in pots was balanced by determining and daily adding of distilled water. Before the 42^{nd} day, water was added to under 0.3 suction pots once a week and then every two weeks and with decrease of amount of pH, once a week; in a way that at the bottom of the pot about 30 mL of solution was sediment. This solution was extracted with 50 mL needle and placed in a polyethylene tube. In stated times, samples were taken from solution of submergence pots and their 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. First, data was normalized and then appropriate conversion was used for abnormal data. Then, means were compared using LSD test with probability level of 5% and graphs were drawn by Excel software.

RESULTS AND DISCUSSION

Some physical and chemical characteristics of soils: Some physical and chemical characteristics of loamy sand and clay loam soils used in this research are represented in Table 1 and 2. Loamy sand soil was a coarse-textured soil with low organic matter and lime. Clay loam soil was an average texture soil, calcareous and low organic matter. Concentration of Fe, Mn and extractable Zn in loamy sand soil was less than clay loam.

Extractable Zn with extractor: Variance analysis shows the main effect of soil, main effect of moisture, fertilizer, time and their interaction was meaningful in probability level of 1% (Table 3). Comparing means show in urea containing levels and submergence and un-submergence state, first extractable Zn decreases and then increases (Fig. 1 to 3). Initial decrease of extractable Zn in surface with urea can be related to increase of Fe and Mn ions during initial incubation (un-submergence and submergence) and their antagonist effect with Zn. Decreasing availability of Zn in soil after submergence can be due to decrease of Redox potential and increasing solubility of phosphorous and their antagonistic effect on Zn (Sajwan and Lindsay, 1986). Increasing redox potential of extractable Zn increases with DTPA, which can be due to oxidation of Fe, Mn and their sedimentation as Fe $(OH)_3$and MnO_2. Reduction in concentration of extractable Zn when using ammonium fertilizer is reported by He *et al.* (1999). On the other hand, increase of pH in soil in different levels of urea, decreases transferred Zn bonds with oxide and concentration of extractable Zn (Fig. 5 and 6). McBride and Blasiak (1979) also observed increasing every unit of pH in range of 5-7, decreases concentration of dissolved Zn 30 times. Weil and Holah (1989) also reported similar results. Extractable Zn of clay loam soil increased after 5 days of incubation in submergence state in all three levels of fertilizer and then was fixed (Fig. 2), while concentration of Zn decreased after 5 days of submergence in two control level and 200 mg N/kg and then increased (Fig. 4). Towfighi and Najafi

Table 1: 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 2: 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

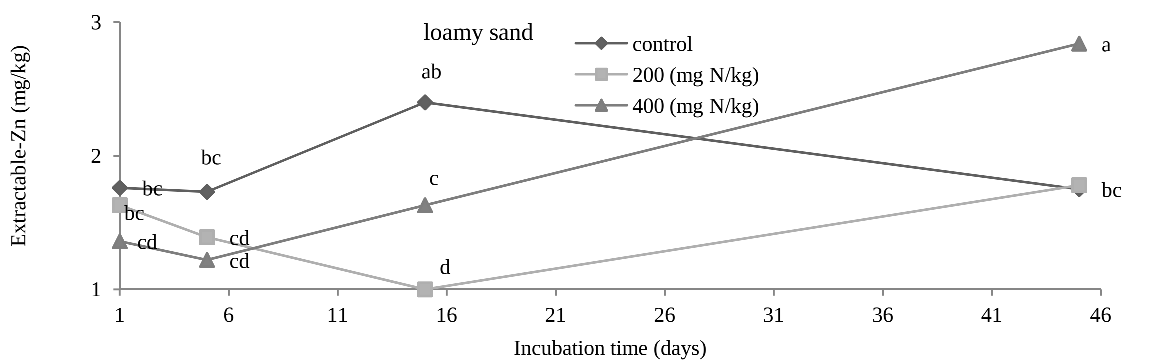

Fig. 1: Interaction effect of incubation time and urea levels on extractable Zn in loamy sand soil

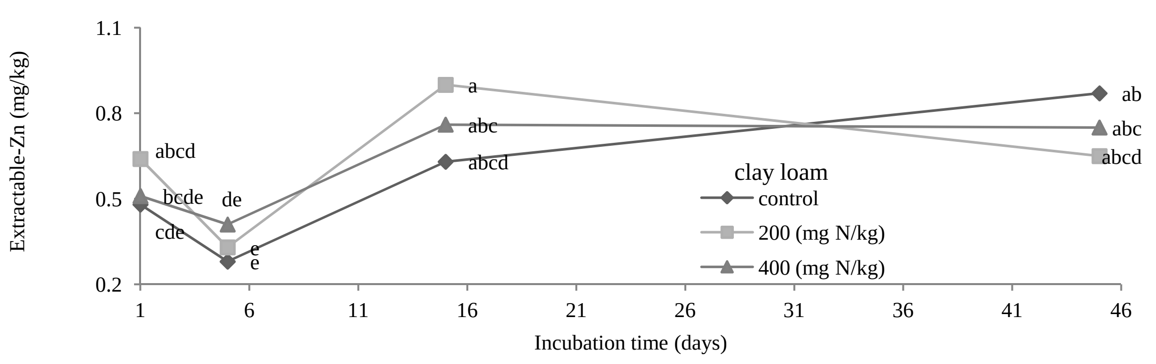

Fig. 2: Interaction effect of incubation time and urea levels on extractable Zn in clay loam soil

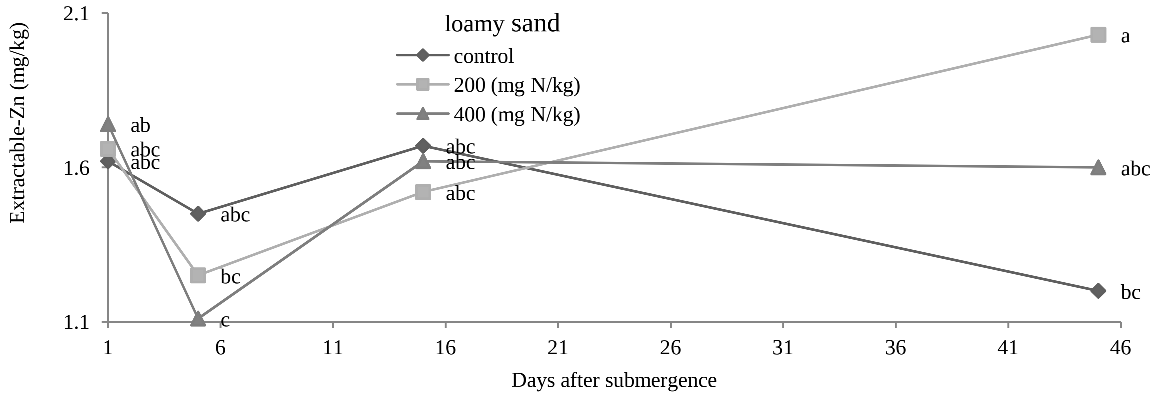

Fig. 3: Interaction effect of submergence time and urea levels on extractable Zn in loamy sand soil

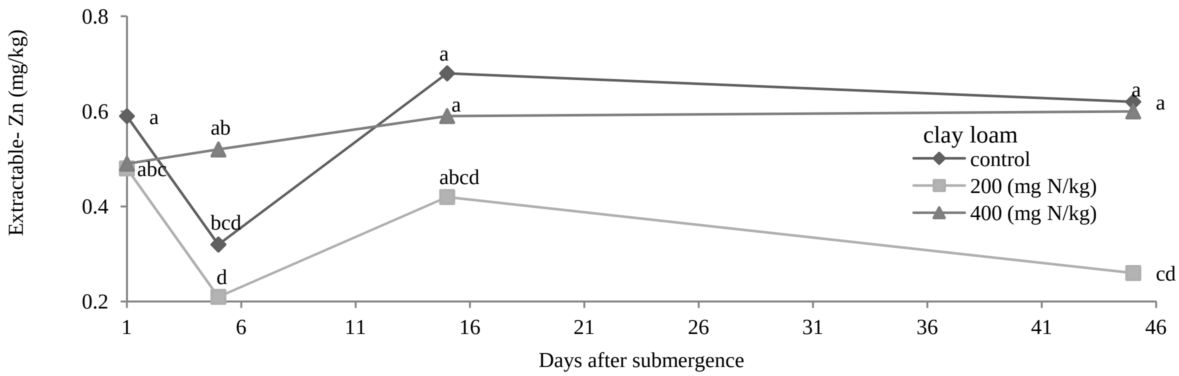

Fig. 4: Interaction effect of submergence time and urea levels on extractable Zn in clay loam soil

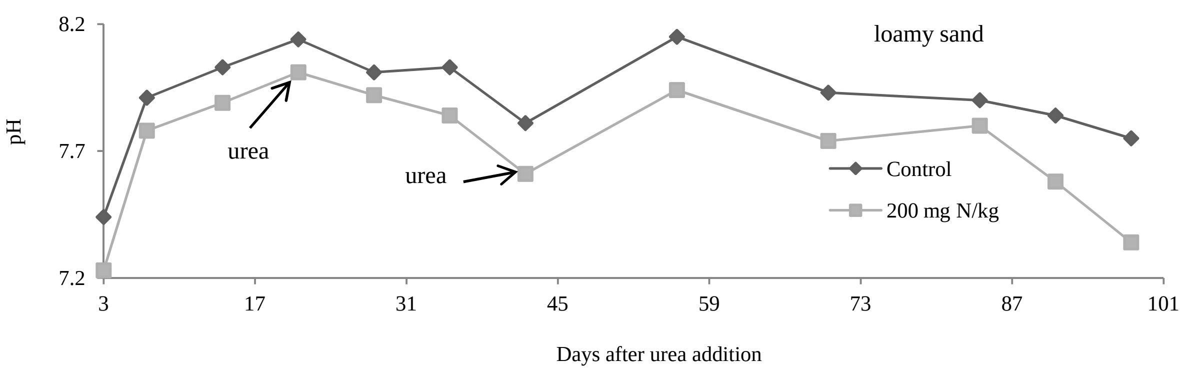

Fig. 5: Effect of urea levels on pH changes of loamy sand in unsubmergence condition

(2002) observed that dissolved Zn in both acidic and alkaline calcareous soils decreased up to 7 days of submergence, then increased and after that again decreased. Misra *et al.* (1989) observed that extractable Zn with DTPA of soil decreased after submergence and after about 20 days it was fixed. In submergence state, availability of Zn decreased by increase of pH (Fig. 7). Zn sediments in acid soils as $Zn(OH)_2$or as Zn S in sodium and calcareous soils. However, Zn extremely adsorbs by $CaCO_3$, $MgCO_3$ and Fe and Mn oxides. Increasing concentration of organic acids produced on submergence state limits absorption of Zn. In anaerobic conditions, insoluble Zn phosphate forms (Dobermann and Fairhurst, (2000); Dutta *et al*. (1989) stated that decreasing availability of Zn in acid soils up to neutral condition is due to their sedimentation

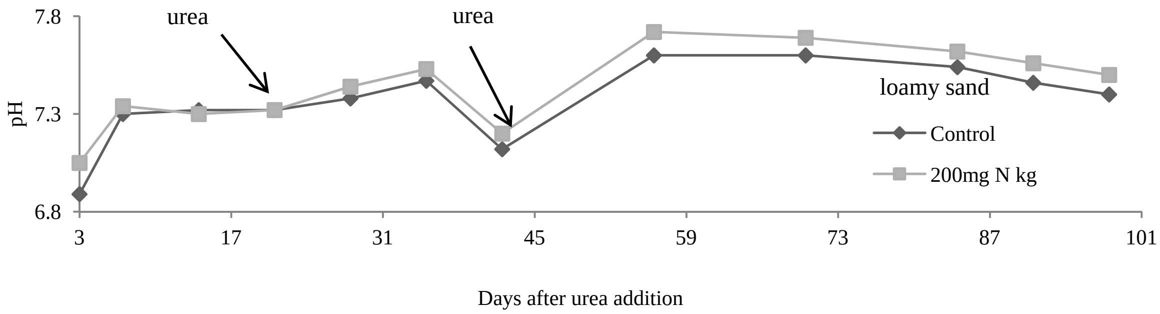

Fig. 6: Effect of urea levels on pH changes of loamy sand in submergence condition

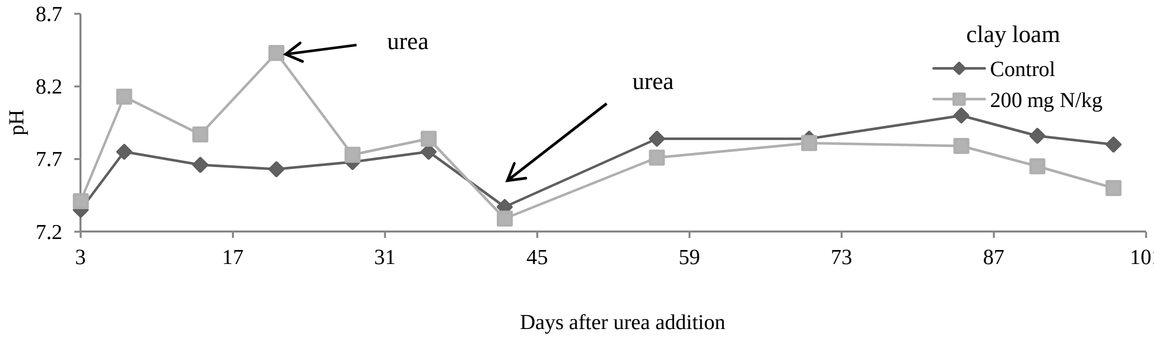

Fig. 7: Effect of urea levels on pH changes of clay loam in submergence condition

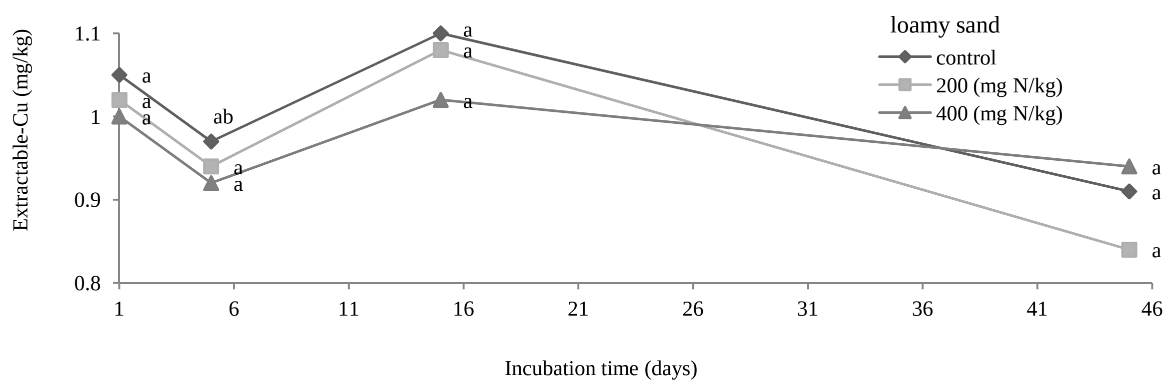

Fig. 8: Interaction effect of incubation time and urea levels on extractable Cu in loamy sand soil

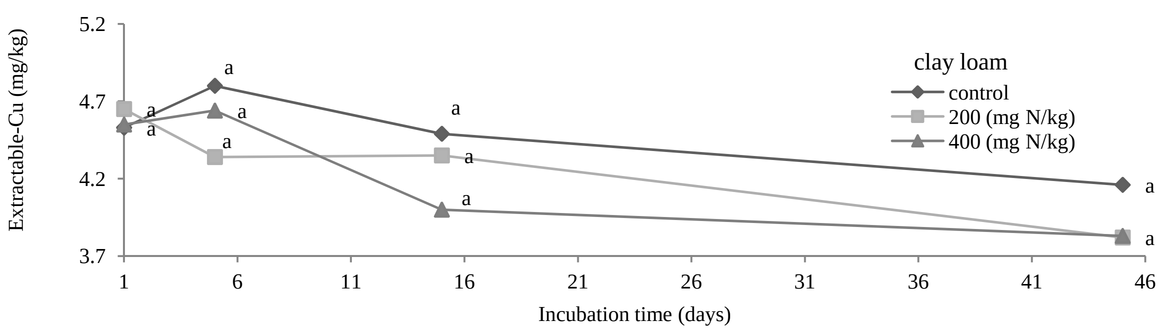

Fig. 9: Interaction effect of incubation time and urea levels on extractable Cu in clay loam soil

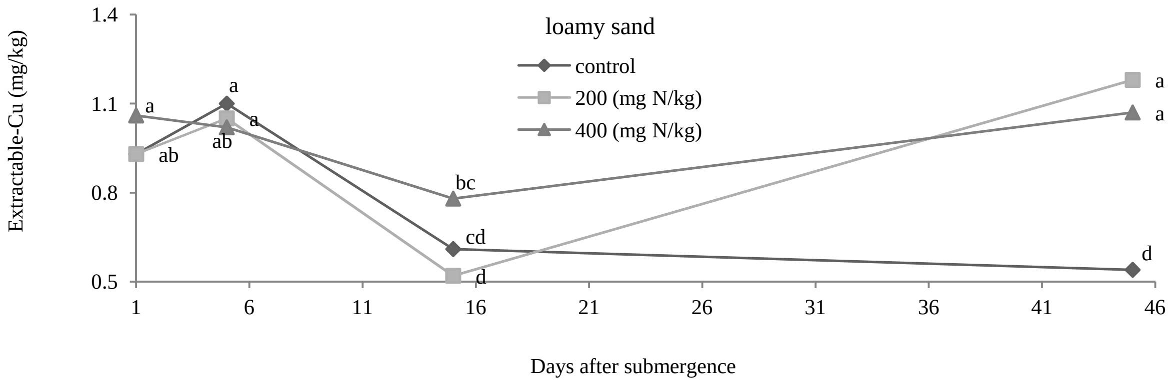

Fig. 10: Interaction effect of submergence time and urea levels on extractable Cu in loamy sand soil

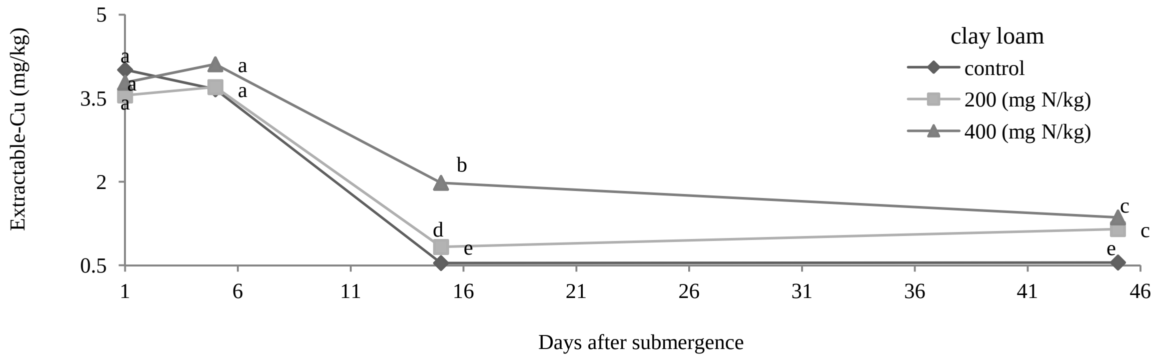

Fig. 11: Interaction effect of submergence time and urea levels on extractable Cu in clay loam soil

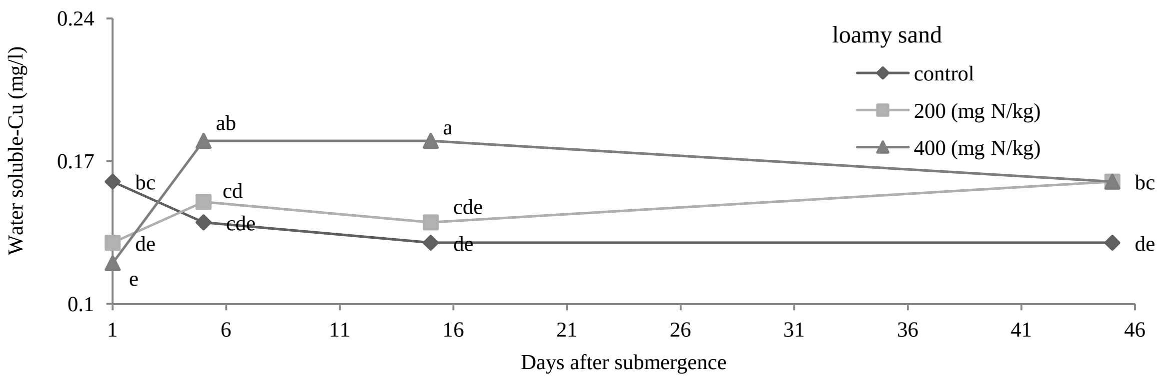

Fig. 12: Interaction effect of submergence time and urea levels on water soluble Cu in loamy sand soil

as hydroxide, carbonate and sulfide. Using 3 g/kg calcareous in Alfisolssoil Naik and Das (2007) represented that concentration of extractable Zn decreases after 35 days of incubation in three moisture condition (continuous and intermittent saturation, submergence). They believe this decrease is due to formation of insoluble Zn or sedimentation of Zn with calcareous. Very little amount of Zn makes it difficult to measure concentration of dissolved Zn in water.

Extractable Cu with extractor: Variance analysis shows the main effect of soil, main effect of moisture, fertilizer, time and their interaction was meaningful in probability level of 1% (Table 3). Figures 8 and 9 show in both soils, there is no meaningful difference in extractable Cu between different fertilizer levels in un-submergence state. This may be due to low amount of nativecopper in both soils. Mitchell *et al.* (1999) reported using urea has no meaningful effect on extractable Cu. Malhi *et al.* (1998), Dortzbach *et al.*

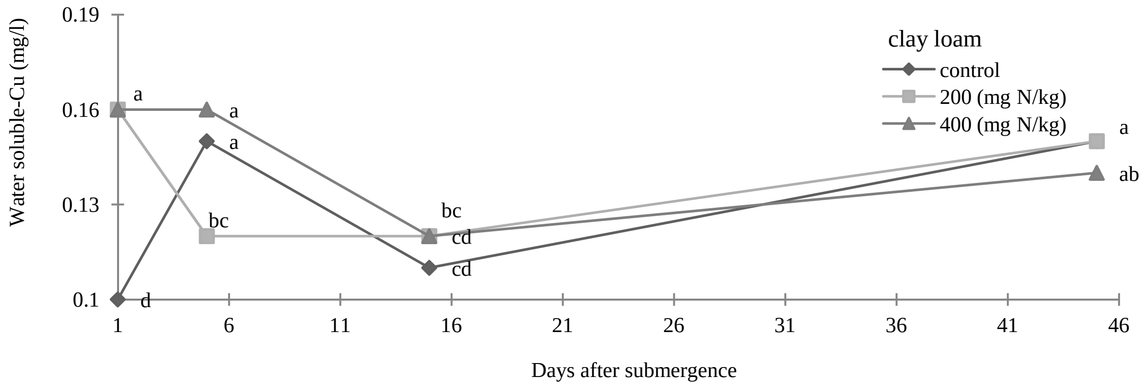

Fig. 13: Interaction effect of submergence time and urea levels on water soluble Cu in clay loam soil

Table 3: Variance analysis of the effect of soil type, moisture level, urea and incubation time on extractable Zn and Cu using extractor

		Mean squares	
Treatments	df	Zn	Cu
Soil	1	3.2**	5.53**
Moisture	1	0.05**	0.91**
Soil×moisture	1	0.004 n.s	0.6**
Urea	2	0.02**	0.03**
Soil×urea	2	0.004 n.s	0.005*
Moisture×urea	2	0.001 n.s	0.05**
Soil × moisture×urea	2	0.05**	0.009**
Time	3	0.09**	0.26**
Soil×time	3	0.01**	0.14**
Moisture×time	3	0.02**	0.19**
Soil×moisture×time	3	0.005 n.s	0.09**
Urea×time	6	0.008*	0.01 **
Soil×urea×time	6	0.02**	0.004 n.s
Moisture×urea×time	6	0.003 n.s	0.02**
Soil×moisture×urea×time	6	0.009**	0.005**
Error	48	0.003	0.002
CV (%)		5.19	3.29

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

Table 4: Variance analysis of the effect of soil type, urea and submergence time on dissolved micronutrients in water

Treatments	df	Mean squares Cu
Soil	1	0.12**
Urea	2	0.18**
Soil×urea	2	0.007 n.s
Time	3	0.08**
Soil×time	3	0.08**
Urea×time	6	0.05*
Soil×urea×time	6	0.14**
Error	24	0.01
CV (%)		8.19

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

(2010), Saleh *et al*. (2012) reported similar findings. Extractable Cu of both soils decreased 15 days after submerging in all three levels of fertilizer, but again increased in loamy sand soil and in 200 and 400 mg N/kg (Fig. 10 and 11). Najafi *et al*. (2012) reported absorbable Cu in both loamy sand and clay loam soils decreased 30 days after submergence, which is in accordance with findings of Dutta *et al*. (1989) and Saha *et al*. (1992). Dutta *et al*. (1989) states reduction has meaningful correlation with initial pH of the soil, initial amount of extractable Cu with DTPA, organic carbon and clay. Saha and Mandal (1998) reported submerging soil, changes active forms of Cu such as soluble, exchangeable and organic complexes to inactive form, therefore, availability of Cu decreases after submergence. Singh *et al*. (1982) reported extractable Cu with DTPA of a calcareous soul with loamy sand texture increases up to 4 weeks after submergence, then decreases up to 12th week after submergence. Initial increase of extractable Cu with DTPA relates to decrease of pH of soil after submergence. Sahrawat (2005) reported submerging soil increases absorbable Cu. These results show the effect of submergence on absorbable Cu of plant depends on type of soil and condition of research.

Dissolved Cu in water: Variance analysis shows the main effect of soil, main effect of moisture, fertilizer, time and their interaction was meaningful in probability level of 1% (Table 4). Comparing means indicate dissolved Cu in water meaningfully increases when using fertilizer; low amount of it was in control level and the high amount of it was in 400 mg N/kg level(Figures 12 and13). Microbial immobilization and antagonist effect of increasing concentration of Fe, transferrable Mn and phosphorous are proposed as probable causes of decreasing amount of Mn and Zn (Haldar and Mandal, 1979). As states above, due to rapid hydrolyzing of urea at initial times of submergence and increasing concentration of extractable and dissolved Fe and Mn during these times and low native Cu of soil, concentration of dissolved Cu decreased. After long term using chemical fertilizers (nitrogen fertilizer with urea source), Prasad and Sinha (1982) reported increasing amount of urea decreases availability of Cu at the soil. Submergence of soil decreases concentration of dissolved Cu and Zn in water. Decreasing solubility of Cu can be due to its change to insoluble sulfides (Ponnamperuma, 1985).

CONCLUSION

Results of the study show the concentration of extractable Cu and Zn in un-submergence state is more than submergence condition. Using urea increases concentration of dissolved Cu. Using both level of urea in submergence and un-submergence condition, extractable Zn of loamy sand soil first decreases and then increases. In clay loam soil with 200 mg N/kg, extractable Zn first decreases and then increases. Changes of extractable Cu of both soils in three levels of fertilizer and un-submergence condition are not meaningful but in submergence state, urea increases extractable and dissolved Cu of both soils. In general, urea affects solubility of micronutrient elements which differs in various times of incubation. It's worthy to note that the effect of urea is not meaningful on changes of Cu in both soils in un-submergence condition, which is due to low amount of native Cu in soils. However, the effect of moisture level on availability of elements at clay loam soil was different due to calcareous, moderately heavy texture and high buffering power.

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