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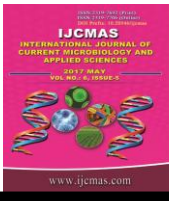

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Effect of Copper Contamination on Soil Biochemical Activity and Performance of Rice (*Oryza sativa* L.)

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ABSTRACT

Keywords

Copper,
Rice, Biochemical activity, Nutrient concentration.

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A pot culture experiment was carried out to determine the effect of soil contamination with CuSO$_4$ 5H$_2$O in different concentrations on the activity of soil enzymes (Dehydrogenases, Urease, Acid and Alkaline Phosphatase) and dry matter yield of rice. The experiment was conducted in completely randomized design comprising 6 levels of Cu (0, 50, 100, 150, 200 and 250 mg kg^{-1} soil). Soil contamination with Cu also had a negative effect on the dry matter yield and yield attributes of rice. Toxic activity of Cu on rice appeared at the lowest dose (100 mg kg^{-1}), and higher doses of Cu were found to intensify the effect. The enzyme activity in the soil samples was determined at 30, 60, 90 DAS and at harvest. The enzyme activity increased up to 30 DAS which later decreased to harvest. The results indicated that, soil contamination with CuSO$_4$ 5H$_2$O of 100, 150, 200 and 250 mg Cu kg^{-1} soil significantly inhibited the activity of dehydrogenases, urease, acid and alkaline phosphatases. The soil enzymes can be arranged in terms of their sensitivity to Cu as follows: dehydrogenases > urease > alkaline phosphatase > acid phosphatase. Dehydrogenases and urease appeared to be better indicators of soil contamination with Cu, as their activity was more strongly inhibited by Cu than the activity of phosphatases.

Introduction

Copper (Cu) is an essential element for regular functioning of organisms. It plays significant role in number of physiological processes like photosynthetic and respiratory electron transport chains, N fixation, protein metabolism, cell wall metabolism, anti oxidant activity, fatty acid metabolism and harmone perception. However, excessive amounts of Cu in the root zone inhibit growth, chlorosis of leaves and limited germination of seeds. The higher absorption of Cu contributes to metabolism disturbances, damages to plasma membrane permeability, membrane integrity and induces general symptoms of senescence.

Cu is extensively used in agriculture in the form of Cu containing fertilizers, fungicides, bactericides, algicides and in the form of metal contaminated composts, sewage sludge as well as feed additive in antibiotics, drugs, growth promoters etc. Its abundance in the earth crust (24-55 µg g^{-1}) and soil (20-30 µg g^{-1}) makes it pollution problem in most agricultural soils (Pendias and Pendias, 2001). Cu enter into the soil through various sources affecting soil microbial properties responsible for nutrient recycling and enzyme activities within soil-plant ecosystem. Keeping in view the effect of Cu contamination on soil biochemical activity and growth of rice, an experiment was conducted to determine the effect of soil contamination with $CuSO_4$ $5H_2O$ in different concentrations on the activity of soil enzymes (Dehydrogenases, Urease, Acid and Alkaline Phosphatase) and dry matter yield of rice.

Materials and Methods

A pot culture experiment was conducted in earthen pots contains 5 kg of well mixed air dried red sandy loam soil. The rice *var*. BPT 5204 used as test crop. Carefully selected uniform sized seeds were directly sowed in each pot. The experiment consisting of six treatments comprising 6 levels of Cu (0, 50, 100, 150, 200 and 250 mg kg^{-1} soil). Cu treatments were given through addition of varying amounts of $CuSO_4.5H_2O$. The recommended doses of fertilisers were applied uniformly to all the treatments. The treatments were replicated five times in a completely randomized design. The experimental soil is sandy loam in texture, slightly alkaline (pH 7.2) in reaction, non saline (0.18 dSm^{-1}), low in organic carbon (0.43 percent) and available N (196.5 kg ha^{-1}), medium in available P_2O_5 (29.21 kg ha^{-1}) and K_2O (293.5 kg ha^{-1}) and having sufficient amounts of micronutrients.

Plant samples collected at harvest was dried in an oven and analyzed the contents of N, P, K, S, Fe, Mn, Zn and Cu. Dry weight of root and shoot was determined. Oven dried plants were digested in appropriate acid mixtures and the nutrient contents were measured. Using the acid digest, nitrogen was determined by micro-Kjeldahl method and phosphorus was determined by vanadomolybdate method measuring the absorbance at 460 nm by spectrophotometer. K, Na, Ca and Mg were determined by flame photometer. Fe, Mn, Zn and Cu were determined by atomic absorption spectrophotometer (AAS). The statistical analysis of the experimental data was carried out as per the procedure given by Gomez and Gomez (1984).

The enzyme activity in the soil samples was determined at 30, 60, 90 DAS and at harvest. Urease activity was assayed by qualifying the rate of release of NH_4^+ from the hydrolysis of urea as described by Tabatabai and Bremner (1972) but with some modifications as suggested by Sankara Rao (1989). Dehydrogenase activity was assayed by quatifying the mg of TPF (2, 3, 5-tri-phenyl formazon) produced and exposed as $g^{-1}soil^{-1}d^{-1}$ as described by Casida *et al.*, (1964). The acid and alkaline phosphatase activity was assayed by quantifying the amount of P-nitrophenol released and expressed as µg of P-nitrophenol released $g^{-1}soil^{-1}d^{-1}$ as described by Tabatabai and Bremner (1969).

Results and Discussion

Dry matter yield and yield attributes of rice

The results indicated that application of Cu slightly increased the root and shoot dry weight at lower concentrations, while excess Cu reduced the biomass (Table 1). Moreover, high concentrations of Cu, the root and shoot elongation was poor with a concomitant decrease in root and shoot drymatter (Bouazizi *et al.*, 2008; Ahsan *et al.*, 2007). Significant increase in the growth, possibly due to Cu is required by plants in trace amount (Reichman, 2002). The inhibitory action of excess Cu in root and shoot length may be due to reduction in cell division, toxic effect of heavy metal on photosynthesis, respiration and protein synthesis.

Plant nutrient concentration (%)

The effect of Cu on various micronutrient contents like NPK of rice plant at harvest indicated that, nutrient contents increased at lower level (50 mg kg^{-1}) and decreased to higher level (100 to 250 mg kg^{-1}). The inhibitory effect of Cu on macronutrient content of rice plant could be attributed to poor development of roots, reduced rate of protein metabolism which results in decreased uptake of macronutrients from the soil. High concentration of Cu suppresses the P metabolism by lowering the content of inorganic P. The decrease of K content of rice due to elevated levels of Cu may be attributed to deterioration of physiological state of the plant which intern reduction in K uptake. The decrease in potassium content of rice due to elevated level of Cu is in conformity with the reports of Lidon and Henriques (1993) and Ouzounidou (1994).

Increased Cu content of soil slightly decreased the micronutrient content of rice (Table 2). However lower levels of Cu (50 mg kg^{-1}) increases the Fe, Mn and Zn content of rice plant over control. Excess Cu antagonistically affects the translocation of Fe, and Zn from the stem to the leaves and increased the competition of Cu with Fe, Mn and Zn. The decrease in Mn content may be due to increased competition of Cu with Mn for transport sites in plasma lemma (Wang *et al.*, 2009). Application of Cu did not affect concentration of Zn, but higher levels causes antagonistic effect.

Soil enzyme activities

The results indicated that the enzyme assayed at different growth stages of rice showed that there was increase in enzyme activity up to 30 DAS which later decreased to harvest. Soil contamination with $CuSO_4$ $5H_2O$ of 100, 150, 200 and 250 mg Cu kg^{-1} soil significantly inhibited the activity of dehydrogenases, urease, acid and alkaline phosphatases. The soil enzymes can be arranged in terms of their sensitivity to Cu as follows: dehydrogenases > urease > alkaline phosphatase > acid phosphatase.

Table.1 Effect of Cu on dry matter yield and yield attributes of rice

Cu added in the soil (mg kg^{-1})	Dry matter yield (g $hill^{-1}$)		No. of effective tillers $hill^{-1}$	No. of matured grains per $panicle^{-1}$
	Root	Shoot		
0	1.53	4.35	06	163
50	1.74	4.40	06	154
100	1.31	4.11	04	139
150	1.08	2.74	03	83
200	0.32	1.59	01	70
250	0.21	1.35	-	-
CD (0.05)	0.022	0.04	1.33	9.95
S.Ed±	0.01	0.01	0.67	4.89

Table.2 Effect of Cu on nutrient content of the rice plant at harvest

Cu added in the soil (mg kg^{-1})	**N**	**P**	**K**	**Cu**	**Mn**	**Fe**	**Zn**
	(%)			**(mg kg^{-1})**			
0	1.171	0.424	2.332	2.81	33.01	74.97	12.24
50	1.296	0.487	2.350	3.34	37.27	78.74	13.25
100	0.874	0.377	2.246	5.31	29.71	72.21	8.51
150	0.796	0.316	1.983	8.61	23.64	63.65	7.82
200	0.713	0.211	1.829	11.51	21.56	61.38	7.17
250	0.606	0.168	1.666	13.06	16.66	41.48	6.42
CD (0.05)	0.102	0.048	0.109	0.343	3.25	4.38	0.69
S.Ed±	0.046	0.022	0.049	0.156	1.48	1.99	0.32

Fig.1 Effect of levels of Cu on urease enzyme activity (µg of NH_4^+-N released g^{-1} soil h^{-1}) of soil at 30, 60, 90 DAS and at harvest of rice

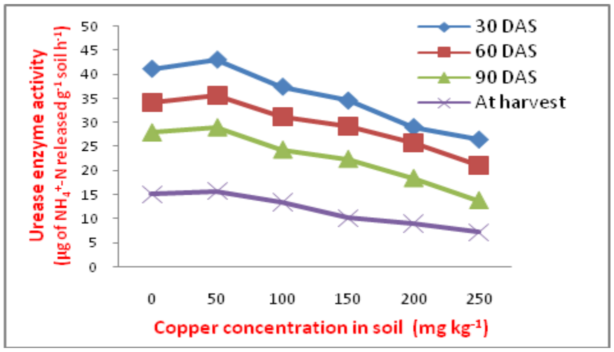


Fig.2 Effect of levels of Cu on dehydrogenase activity (µg of TPF produced g^{-1} soil d^{-1}) of soil at 30, 60, 90 DAS and at harvest of rice

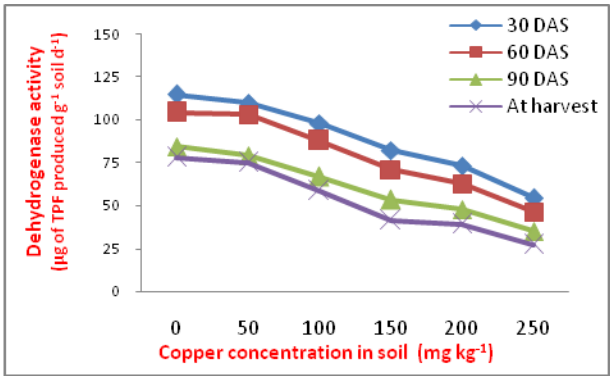


Fig.3 Effect of levels of Cu on acid phosphatase activity (µg of PNP released g^{-1} soil h^{-1}) of soil at 30, 60, 90 DAS and at harvest of rice

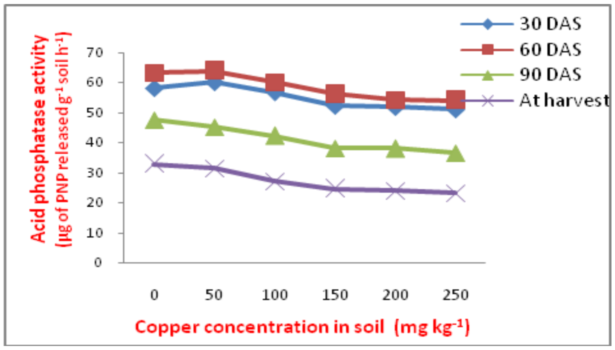
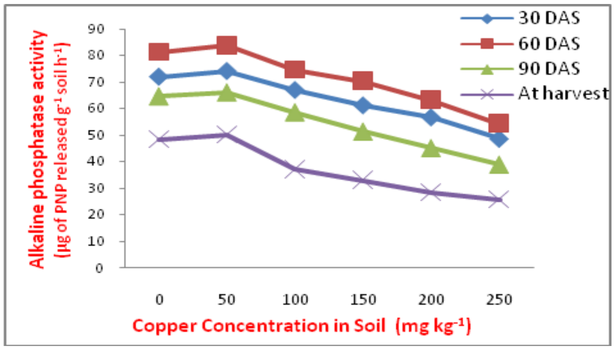


Fig.4 Effect of levels of Cu on alkaline phosphatase activity (µg of PNP released g^{-1} soil h^{-1}) of soil at 30, 60, 90 DAS and at harvest of rice



Urease enzyme activity

The enzyme urease is extracellular enzyme secreted by soil microorganisms which catalyses the hydrolysis of urea to ammonia, which subsequently transformed to NH_4^+ and NO_3^-. It is ranged from 21.09 to 35.81, 26.54 to 43.15, 13.86 to 29.02 and 7.27 to 15.24 µg of NH_4^+ released $g^{-1}soil^{-1}h^{-1}$ at 30, 60, 90 DAS and at harvest, respectively (Figure 1). The highest urease activity recorded with application of Cu @ 50 mg kg^{-1} at all the time intervals. The sharp increase in urease enzyme activity at 30 DAS coincide with active growth stage of the crop enhanced root activity, root proliferation and release of extracellular enzyme which resulting in higher rate of mineralisation of nutrients. The results were in conformity with the findings of Sriramachandrasekharan *et al.*, (1997) and Srinivas *et al.*, (2000). However, increasing cu concentration from 100 to 250 mg kg^{-1} decreased the enzyme activity by more than 50 percent due to decreasing population of microorganism like bacteria (Wyszkowska and Kucharski, 2003). Addition of Cu @ 250 mg kg^{-1} has recorded about 62.39, 55.46, 101.7 and 109.2 percent decrease in urease activity at 30, 60, 90 DAS and at harvest, respectively over control.

Dehydrogenase enzyme activity

The enzyme dehydrogenase is intracellular enzyme produced by soil microorganisms involved in degradation of carbohydrates and lipids. It is ranged from 36.48 to 64.67, 44.59 to 75.03, 25.24 to 44.58 and 17.25 to 38.18

µg of TPF produced g^{-1} soil d^{-1} at 30, 60, 90 DAS and at harvest, respectively (Figure 2). The highest dehydrogenase activity recorded in control. Increasing cu concentration from 50 to 250 mg kg^{-1} decreased the enzyme activity by more than 2 times. Addition of Cu @ 250 mg kg^{-1} has recorded about 77.27, 68.26, 76.62 and 121.3 percent decrease in dehydrogenase activity at 30, 60, 90 DAS and at harvest, respectively over control. Dehydrogenase being intracellular enzyme more sensitive to effect of excess Cu compared to other enzymes. Cu is highly toxic to micro organisms if present in excess concentration which consequently changes soil biological equilibrium with adverse effect on both soil fertility, plant development and yield. Excess Cu prevent the formation of red colour development product (TPF) from TTC, there is a biological conversion of TPF to colour less compound results in decrease in dehydrogenase activity (Wyszkowska, 2006).

Acid and alkaline phosphatase enzyme activity

The enzyme phosphatises breaks hemi cellular compounds of organic materials to produce humus and H_3PO_4 making P available to plants. Acid phosphatise activity is ranged from 55.09 to 60.26, 58.09 to 63.89, 40.54 to 47.58 and 27.24 to 32.78 µg of PNP released g^{-1} soil h^{-1} at 30, 60, 90 DAS and at harvest, respectively (Figure 3). Alkaline phosphatise activity is ranged from 48.56 to 74.26, 54.29 to 83.75, 39.08 to 66.02 and 25.89 to 48.55 µg of PNP released g^{-1} soil h^{-1} at 30, 60, 90 DAS and at harvest, respectively (Figure 4). Addition of Cu from 50 to 250 mg kg^{-1} slightly decreased the enzyme activity over control. None of the dose of Cu inhibited the Acid and Alkaline Phosphatase Enzyme Activity more than 20 percent, only two higher doses *i.e.* 200 and 250 of Cu inhibited the activity more than 50 percent. Similar results reported by Wyszkowska (2005).

From these observations it can be concluded that, low Cu concentration had stimulatory effect on growth, dry matter yield and mineral nutrient content of rice. Application beyond these levels (100-250 mg kg^{-1}) adversely affected the growth, dry matter yield and nutrient content. Among the enzymes studied, urease and dehydrogenases appeared to be better indicators of soil contamination with Cu, as their activity was more strongly inhibited by Cu than the activity of phosphatases. Cu is highly toxic to micro organisms if present in excess concentration which consequently changes soil biological equilibrium with adverse effect on both soil fertility, plant development and yield.

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