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Critical Value of Copper for Hydrolytic Enzymes in Soil and Plant Growth and Development in Sugarcane

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Abstract – **A pot culture experiment was conducted using sugarcane variety GT21 and different Cu concentrations. The results showed that the invertase and urease activities increased initially up to certain level and then decreased with increase of total Cu concentration in soil. The maximum activities of enzymes have been recorded in the range of 86.65 mg/kg to 94.15 mg/kg Cu in soil. The plant height, stalk diameter, cane yield, and juice brix increased and attained maximum values at 269.15 mg/kg. At the 269.15 mg/kg concentration of Cu in soils, the total Cu content in cane was observed as 7.82 mg/kg. Our studies revealed that the critical values of Cu in soil ranged between 86.65 mg/kg -94.15 mg/kg, above which the soil enzyme activities decreased significantly. While for sugarcane growth, critical values of Cu in soil was found to be 269.15 mg/kg. Further, soil invertase and urease enzymes may be used as biochemical markers for heavy metal (Cu) pollution in soils.**

Keywords – **Copper (Cu), Critical Value, Hydrolytic Enzyme, Sugarcane.**

I. INTRODUCTION

Currently, the heavy metal pollution in soils has become a great threat to plant's growth and development in many countries. On average, annual emission of Cu has been found about 3.4 million tons in the world [1]. According to statistics, nearly 20 million ha of land was affected from heavy metal pollution in China, which was about 20 percent of Chinese cultivated land. Among heavy metals, Pb, Cd, Cu, and their complexes were the most prominent pollutants [2]. Most of the studies related to effects of Cu pollution on crop growth, yield, and quality are concentrated on vegetables, wheat, corn, rice, etc.[3] [4] [5] [6], and very few reports are on sugarcane. Guangxi occupies more than 65% of sugarcane growing area of China, and accounts for the same percentage of sugarcane and 70% cane sugar yield in China. Most of the sugarcane crops are grown in the dry and sloppy areas [7] which depend on rains for their water requirement. Hang, W.X. et al investigated the status of Cu pollution in the dry slopy areas of 36 counties of Guangxi and found that 5.13% of soil had higher amount of Cu than the normal limits. Nearly 50 thousand ha of sugarcane land in Guangxi come under the risk of Cu pollution [8]. In the present study, a pot experiment was conducted to observe the effects of different concentration of Cu on soil enzymes and sugarcane characteristics, and to find out the critical value of Cu for sugarcane plantations.

II. MATERIALS AND METHODS

A. Experimental Material

A pot culture experiment was conducted in completely randomized block design using the sugarcane variety GT21 (breeded by Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences). The experimental soil was collected from the top layer (0cm -30 cm depth) of a typical sugarcane field in Fusui County, Nanning City, Guangxi province, China. The used soil is described as a red, acidic soil with an initial total Cu content of 44.15 mg/kg. A summary of some important physical and chemical properties of the experimental soil is shown in table 1.

Before planting, experimental soils were treated with N, P, K chemical fertilizer (15-15-15), manufactured by Shandong Ruxi Chemical Industry Ltd). Top dressing fertilizers were used with urea (46.3%N, manufactured by Sichuan Tianhua Limited Share Ltd, China), KCl (60% K2O, imported from Canada, assembled by Sinochem Fertilizer Limited Company, China). CuSO4.5H2O was selected to create 6 different levels of initial total soil copper content as detailed in the experimental design and treatment section.

B. Experimental Design and Treatment

Eight treatments are as follows: (1): no fertilizer (CK1); (2) N, P and K fertilizer (CF: CK2) ; (3) CF+61.65 mg/kg Cu (Cu2); (4) CF+79.15 mg/kg Cu (Cu3); (5) CF+86.65 mg/kg Cu (Cu4); (6) CF+94.15 mg/kg Cu (Cu5); (7) CF+269.15 mg/kg Cu (Cu6); (8) CF+444.15 mg/kg Cu (Cu7). Total Cu concentration in soils of CK1 and CK2 was 44.15 mg/kg. The Cu was applied in the form of $CuSO_4.5H_2O$; N, P, and K fertilizers were given in the form of urea, diphosphate, and potassium sulphate, respectively. Compound fertilizer was used as basal fertilizer. Urea and potassium sulfate were used as top dressing at tillering stage after collecting soil samples. Every pot contained 6 plants which were thinned to three plants per pot after one month of growth. The experiment was carried out from March, 2008 to January, 2009 in greenhouse of research base, GXAAS (an abbreviation of Guangxi Academy of Agricultural Sciences), Nanning, Guangxi, China. The samples were analyzed at the Central Test Laboratory, Agricultural Resources and Environmental Research Institute and Guangxi Crops

Genetic Improvement and Biotechnology Laboratory, GXAAS, Nanning, Guangxi, China.

C. Samples Collection and Analytical Methods

Soil samples were collected using a 30 cm soil sampling tube at seedling, tillering, elongation, and maturing stages in 2008. These soil samples were sent to lab for analysis of enzymes activity and nutrients content. The plant height, stalk diameter, and brix of cane were investigated in December 2008. Sugarcane harvest was done in January, 2009.

Cu content of soil and plant samples were analysed using the method of Lu, R.K. [9]. Total soil Cu: HCl-HNO_3-HF-$HClO_4$ digestion-AAS (an abbreviation of atomic absorption spectrometry). Plant copper: high-temperature carbonization-HCl dissolved-AAS. Soil enzymes activities were measured according to the method of Guan, S.Y. [10]. Invertase: 3, 5-dinitrosalicylic acid colorimetry. Urease: sodium phenol colorimetry.

Data preparation and statistical analysis were done using Microsoft Office Excel 2003 software and DPS8.01 software.

III. Results

A. Soil Enzyme Activities

The data in Fig.1 and Fig.2 showed that the invertase and urease activities increased initially up to certain level and then decreased with increase of Cu content. The maximum activities of enzymes were recorded in the range of 86.65 mg/kg to 94.15 mg/kg total Cu in soil. This trend was observed at almost all the stages of crop growth (Fig.1 and Fig.2).

B. Sugarcane Growth, Yield, and Quality

With increasing concentration of Cu in soil from 44.15 mg/kg to 269.15 mg/kg, the plant height, stalk diameter, cane yield, and juice brix increased and attained maximum values at 269.15 mg/kg. The highest concentration of Cu (444.15 mg/kg) cut down all the parameters significantly. At the 269.15 mg/kg concentration of Cu in soils the total Cu content in cane were observed as 7.82 mg/kg (table 2). This concentration of Cu in sugarcane plants was found to be critical above which all the parameters increased significantly. The data revealed that moderate Cu content (≤269.15 mg/kg) showed positive effects and resulted in fast growth and high productivity of sugarcane. Besides, the concentration of Cu in sugarcane was found less than the standard value (10 mg/kg) of non-pollution agricultural product when amended with total Cu (≤269.15 mg/kg) into the soil [11].

The higher Cu content in soils led to significant decrease hydrolytic enzymes activities in soil [12] [13]. The urease enzyme is most sensitive to changes in Cu content in soil, thus its activity is used as one of the indexes to assess the effect of different nutrient content and other soil pollutants [14]. Our results clearly showed the effect of changes in soil Cu content on urease and invertase activities. Thus, the two enzymes may be used as a biochemical markers for heavy metal (Cu) pollution in the soils.

The excessive Cu content in soil environment is hazardous to the growth and development of plants. High Cu content in soils has been found to decrease the chlorophyll content and the rate of photosynthesis [15], decrease N, P, K uptake [16], activities of SOD, CAT, and POX and increase the proline content [17], and cause membrane damages [18]. These changes affect plant growth and resulted in lower crop yield and productivity. Gao, F.X. et al. found that Cu content 95.73 mg/kg - 141.65 mg/kg in soil was harmful for the growth of maize crop [19]. However, our studies revealed that sugarcane plants can tolerate up to 269.15 mg/kg Cu in soils without affecting their growth and productivity. Thus, the endurance of sugarcane to Cu was higher than that of the maize plants.

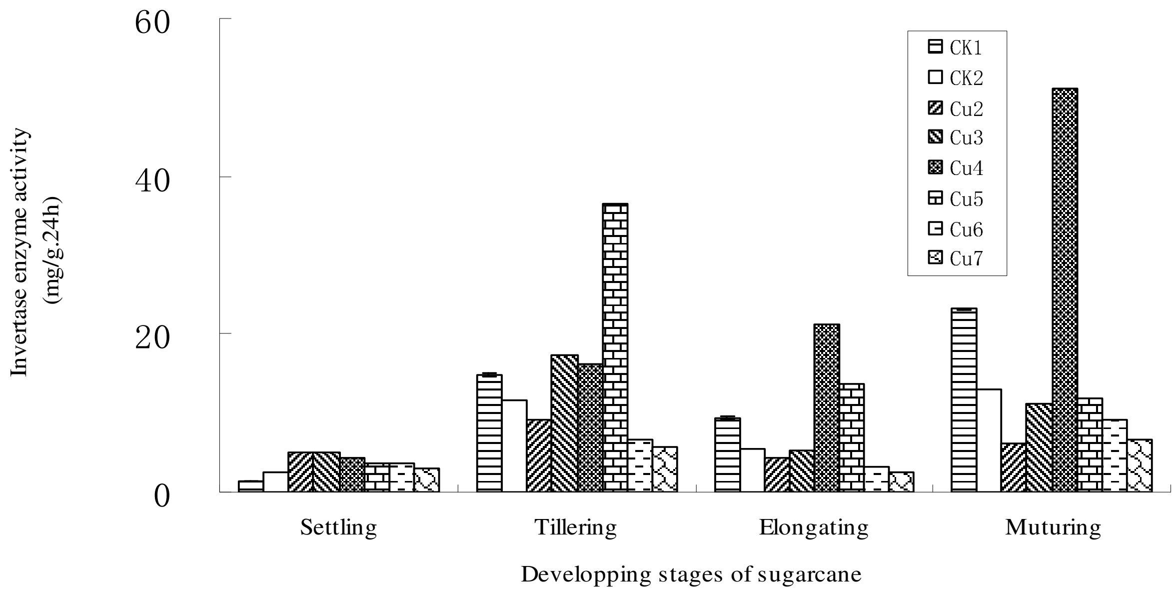

Fig.1. Effect of Cu content on invertase enzyme activity in soil at different stages of sugarcane growth

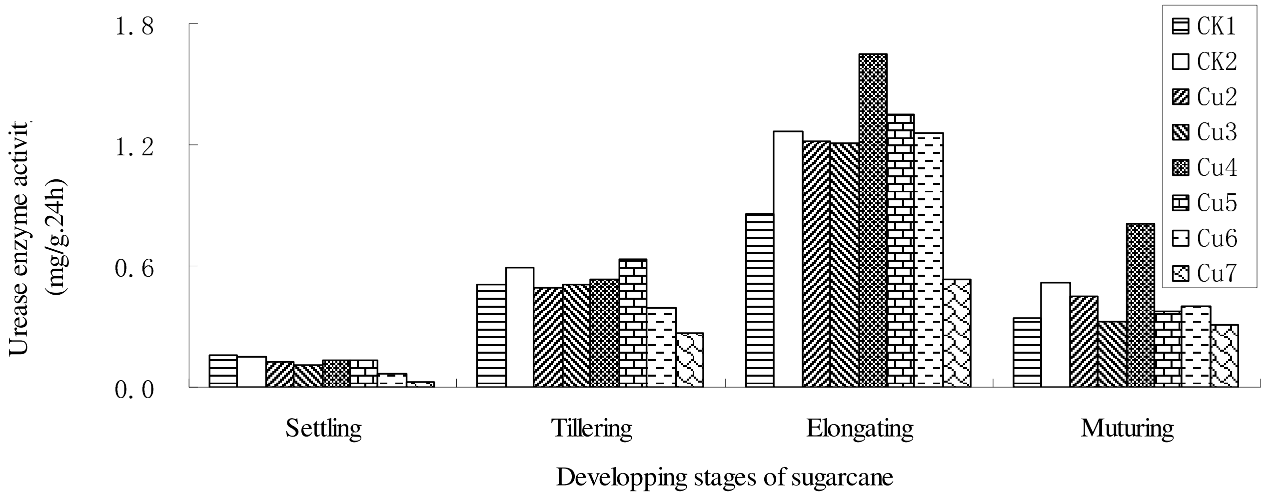

Fig.2. Effect of Cu content on urease enzyme activity in soil at different stages of sugarcane growth

Table 1: Physico-chemical properties of experimental soil

Total N (%)	Total P (%)	Total K (%)	Available N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	pH	Organic matter (%)	Total Cu (mg/kg)
0.08	0.08	0.60	140.00	17.00	126.00	4.51	3.01	44.15

Table 2: Effect of soil Cu content on the growth, yield and quality of sugarcane

Treatments	Plant height (m)	Stalk diameter (mm)	Cane yield (kg/pot)	Juice brix of sugarcane (%)	Cu content in cane stalk (mg/kg)
CK1	1.19±0.09 d B	16.06±0.70 c B	0.64±0.01 e D	18.42±0.29 abc A	4.63±0.01de DE
CK2	1.34±0.13 bcd AB	19.53±1.27 abc AB	1.33±0.01 bc B	15.83±1.15 c A	2.69±0.03 f E
Cu2	1.21±0.22 cd B	16.25±2.84 bc B	0.68±0.03 e D	16.42±0.14 bc A	3.45±0.07 ef E
Cu3	1.26±0.03 bcd AB	16.38±2.35 bc B	0.74±0.02 e D	19.89±0.49 abc A	5.74±0.83 cd CD
Cu4	1.43±0.07 abcd AB	21.39±0.34 abc AB	1.25±0.04 c B	21.08±1.16 abc A	6.08±0.48 bc BCD
Cu5	1.51±0.13 ab AB	22.90±1.99 ab AB	1.43±0.07 b B	21.19±1.65 abc A	6.94±0.23 bc BC
Cu6	1.62±0.05 a A	25.83±1.75 a A	1.89±0.03 a A	22.64±2.93 a A	7.82±0.37 b B
Cu7	1.45±0.06 abc AB	21.61±0.95 abc AB	1.05±0.03 d C	21.74±0.61 ab A	10.06±0.19 a A

Values are the mean of three replicates ± SD; Different capital and small alphabets in same column are representing significant difference at $P \leq 0.01$; and $P \leq 0.05$, respectively.

IV. Conclusion

The present study revealed that the critical values of Cu in soil ranged between 86.65 mg/kg -94.15 mg/kg, above which the soil enzyme activities decreased significantly while for sugarcane plant growth it was found to be 269.15 mg/kg. Further, the soil invertase and urease enzymes may be used as the biochemical markers for heavy metal (Cu) pollution in the soils. The critical value of total Cu in soil, above which sugarcane plant growth was adversely affected, was far higher than GB(with the meaning of national standard) value (50 mg/kg) of total Cu in soil.

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AUTHOR'S PROFILE

Tian-Ming Su

was born in Guigang City, Guangxi, China in 1977. He graduated and earned B. A., M. S. and Ph. D. degrees at Guangxi University in 2001, 2004 and 2009, respectively.

He worked from 2001 until now in Agricultural Resources and Environment Research Institute, GXAAS, China. He was appointed as deputy director since 2007 in this institute. From 2013-2014, he studied as a visiting scholar in University of Illinois at Urbana-Champaign of America.

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