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Response of *Brassica rapa* var. *parachinensis* Grown on Copper Contaminated Oxisol, Inceptisol and Histosol

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ABSTRACT

Copper (Cu) plays a key role in plant physiological and biochemical function but is harmful to plants when in excess. Copper availability is influenced by the mineralogical and chemical properties of soils. High Cu concentration is prominent in soils where vegetables are grown intensively with routine application of fertilizers and pesticides. A factorial pot experiment was carried out to determine Cu critical concentration and toxicity threshold for *Brassica rapa* as well as its soil phase association in Oxisol, Inceptisol and Histosol. Copper sulphate was applied at rates of 0, 5, 10, 15, 20, 30 and 60 mg Cu kg^{-1} soil. The soil Cu critical level in Oxisol, Inceptisol and Histosol was 5.42, 4.67 and 7.79 mg kg^{-1}, respectively; and threshold toxicity level was 12.69, 13.00 and 21.33 mg kg^{-1}, respectively. Height of plants decreased by 50% at a rate of 15 mg Cu kg^{-1} soil for Oxisol and Inceptisol, and at 30 mg kg^{-1} for Histosol. The SPAD value of plant leaves also decreased as the Cu application rate increased starting at 15 mg kg^{-1} in Oxisol and 20 mg kg^{-1} in Inceptisol. The amount of Cu in different soil fractions for both Oxisol and Inceptisol applied with Cu at a rate of 60 mg kg^{-1} was in the order of organic > residual > Fe/Mn oxides > carbonates > exchangeable > water soluble. This study indicated that at 60 mg Cu kg^{-1}, Oxisol had a higher ability to retain Cu in the carbonate, Fe/Mn oxides and organic-bound fractions whereas in Inceptisol, the highest Cu amount was in the residual fraction.

Keywords: *Brassica rapa*, **copper, critical level, phase association, toxicity level, tropical soils**

INTRODUCTION

Soil is a complex heterogeneous medium comprised of various organic and inorganic components, water and air. Interactions among these components control the micronutrients mobility in soil and their availability to plants (Janssen *et al.* 2003; Adriano 2001). Factors like soil pH, cation exchange capacity, organic matter and metal oxides have an influence on the Cu equilibrium shift between the solid and liquid phase, and consequently its concentration in soil solution (Brun *et al*. 1998; Alva *et al.* 2000). Generally, Cu plays an important function in photosynthesis and enzymatic reactions that regulate respiration,

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lignification, and protein synthesis in plants (Awofulu *et al*. 2005; Cohu and Pilon 2010). It is required in small amounts and becomes harmful when present in high concentrations. Garg and Kataria (2009) reported that *Brassica juncea* root and shoot dry weight as well as chlorophyll content decreased with increasing Cu concentration in assay mixtures.

Several studies investigating Cu availability to plants in relation to soil physico-chemical characteristics and Cu phase associations have been carried out (Rodriguez and Ramirez 2005; Gharbi *et al*. 2005). It is important to identify the soil components that primarily control Cu distribution in soils and mechanisms involved in retaining and releasing Cu into the soil solution. Fractionation study aids in predicting Cu distribution in different soils and evaluating the critical and threshold toxic level of Cu in soils. Therefore, this study set out to evaluate *Brassica rapa* growth response and to determine the Cu critical, threshold toxic level of soil in Cu contaminated Oxisol, Inceptisol and Histosol; and to evaluate the Cu phase associations in the Oxisol and Inceptisol.

METHODOLOGY

Soil Physico-chemical Analysis

Uncultivated Munchong, Selangor Series soils and peat (0-20 cm depth) were collected around Selangor area to represent the Oxisol, Inceptisol and Histosol, respectively. The soils were air-dried, sieved through a 2-mm sieve and analysed for bulk density (McIntyre and Loveday 1974), porosity (Tan 2005), soil texture (Gee and Bauder 1986), cation exchange capacity (Thomas 1982), total organic carbon and soil organic matter (Walkley and Black 1934) and Fe, Mn and Al oxides content (Mehra and Jackson 1960).

Pot Experiment

A factorial (3 soils x 7 rates of Cu) pot experiment was carried out at Field 10, Universiti Putra Malaysia (02˚ 99093'N, 101˚ 71380'E). Munchong series soil and peat were limed to pH 5.5 and Cu was applied to the soil in the form of copper sulphate solution at rates of 0, 5, 10, 15, 20, 30 and 60 mg Cu kg^{-1} soil with three replications. The soils were incubated for two weeks, followed by seeding of *Brassica rapa* and application of compound fertilizer 15:15:15 at 0.75 g kg^{-1} soil. A total of 63 experimental units were arranged in Randomized Complete Block Design (RCBD). Plant height and SPAD reading (SPAD 502Plus) of leaves were recorded before harvest. Plants were harvested after 30 days of sowing and oven-dried at 60°C until constant weight was achieved.

Copper Fractionation

Soil samples were air-dried and sieved to 250 µm. Copper fractionation was carried out using sequential extraction procedure of Tessier *et al.* (1979) and modified by Salas *et al.* (1998), Yang and Kimura (1995) and Chlopecka *et al*. (1999) to separate soil Cu into the following fractions: water soluble, exchangeable,

carbonate-bound, Fe/Mn oxides-bound, organic and residual fractions (Table 1). Five grams of soil were placed into a falcon tube and mixed with reagents in accordance to the types of fraction. Samples were centrifuged at 3000 rpm for 20 min after each step; the supernatant was then decanted and analyzed for Cu concentration using atomic absorption spectrophotometry (Perkin Elmer Analyst 400).

TABLE 1
Sequential extraction procedure

Fraction	Procedure
Water soluble	20 mL distilled water, shaken for 2 h using reciprocating shaker
Exchangeable	20 mL of 1M magnesium chloride, shaken for 2 h
Carbonate-bound	20 mL of sodium acetate (adjusted to pH 5.0 using acetic acid), shaken for 2 h
Fe/Mn oxides-bound	20 mL hydroxylamine hydrochloride in 25% acetic acid (v/v), occasionally agitated in water bath (65°C, 8 h)
Organic	2 mL 0.02M nitric acid, 5 mL of 30% hydrogen peroxide (adjusted to pH 2 by 0.1M nitric acid), agitated occasionally in water bath (65°C, 5 h), 3 mL of 30% hydrogen peroxide (continued heating for 7 h), 5 mL of 3.2M ammonium acetate in 20% (v/v) nitric acid, made up to 20 mL with distilled water and shaken for 30 min
Residue	Soil residue was mixed with 50 mL of aqua regia (HCl: HNO_3), heated at 90°C for 2 h, made up to 100 mL with distilled water

Data Analysis

Regression analysis was used to determine the relationship between Cu levels and plant yield, height and SPAD reading of leaves. Data of Cu fractions in Oxisol and Inceptisol were subjected to analysis of variance and mean separation using Student-Newman-Keul's method.

RESULTS AND DISCUSSION

Soil Physico-chemical Properties

A comparison showed Oxisol to have more sand while Inceptisol had more silt. All soils were acidic. Oxisol had the lowest CEC values due to the presence of kaolinite and gibbsite. In comparison to Oxisol, a higher CEC in Inceptisol could be due the presence of 2:1 clay minerals and silt. The Histosol had the highest CEC (43.14 $cmol_{(+)}$ kg^{-1}) due to a large amount of organic matter as the main material of peat (Table 2).

TABLE 2
Physico-chemical and mineralogical properties of Oxisol, Inceptisol and Histosol

Soil properties	Soil Order		
	Oxisol	Inceptisol	Histosol
*USDA Taxonomy	Haplic Hapludox	Typic Tropaquept	Typic Haplohemist
Series name	Munchong	Selangor	-
Bulk density (g cm^3)	1.06	1.03	0.34
Porosity (%)	53.91	55.22	
Texture	Clay	Clay	Organic
% Sand	26.23	0.62	-
% Silt	7.86	31.40	-
% Clay	65.79	67.93	-
pH (H_20)	4.83	5.10	3.13
CEC ($cmol^+ kg^{-1}$)	7.49	14.79	43.14
Total organic carbon (%)	2.40	1.99	55.58
Total soil organic matter (%)	4.13	3.42	95.60
Free oxides (mg g^{-1})			
Fe	33.25	9.03	-
Al	7.87	1.14	-
Mn	0.01	0.26	-
**Main clay mineral type	Kaolinite, gibbsite	Kaolinite, illite	-

*Paramananthan (2000); **Akma *et al.* (2009)

Critical Level and Toxicity Threshold Limit for Brassica rapa

The yield response of the test crop on Oxisols, Inceptisol and Histosol was quadratic: $y=0.83+0.30x–0.02x^2$, $y=1.73+0.32x–0.02x^2$ and $y=1.68+0.20x–0.01x^2$, respectively (*Fig. 1*). The maximum yield of *Brassica rapa* grown in Oxisol, Inceptisol and Histosol was calculated from the equation. The critical soil Cu level required for 90% maximum yield and the toxicity threshold limits (10% reduction in yield) (Guo *et al.* 2010) was obtained from the maximum yield (Table 3).

Plant growth response could be affected by the mobile fraction concentration of Cu present at the time of harvest. Water soluble and exchangeable fractions together make up the mobile fractions. The results of Cu fractionation showed that the Inceptisol contained a higher summation of water soluble and exchangeable fraction (0.260–0.271 mg kg^{-1}) compared to Oxisol (0.164–0.168 mg kg^{-1}) at Cu rates of 0 to 5 mg kg^{-1}. In other words, there was a higher Cu concentration in the soil solution of Inceptisol than that of Oxisol allowing for optimal growth of *Brassica* and tolerance at the aforesaid levels. There was no significant difference of water soluble and exchangeable fractions from 10 to 15 mg Cu kg^{-1} in Oxisol and Inceptisol. This explains the toxicity levels of Oxisol and Inceptisol which were 12.69 and 13.00 mg Cu kg^{-1}, respectively. As a result, the critical Cu level and toxicity threshold limits in Inceptisol was the lowest, followed by the Oxisol and Histosol. It has conclusively been shown that *Brassica chinensis* yield decreases with increasing exchangeable fraction of Cu in soil amended with

composted sludge (He *et al*. 2007). These findings support the assumption that the growth of *Brassica rapa* in the present study was likely to be affected by available soil Cu concentrations.

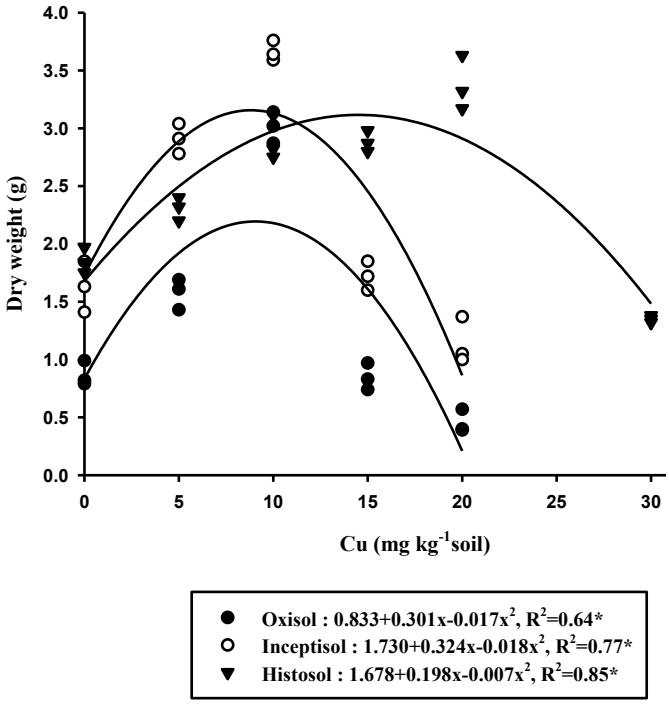


Fig. 1: Yield response to increasing Cu concentration in Oxisol, Inceptisol and Histosol

TABLE 3
The critical and toxicity threshold level of Cu for *Brassica rapa*

Soil	Equation	Critical level	Toxicity level
		---------- mg kg^{-1} ----------	
Oxisol	$y = 0.83+0.30x-0.02x^2$	5.42	12.69
Inceptisol	$y = 1.73+0.32x-0.02x^2$	4.67	13.00
Histosol	$y = 1.68+0.20x-0.01x^2$	7.79	21.33

Peverill *et al*. (1999) briefly explained that the quadratic relationship between a plant response and applied nutrients depicts a large plant yield response up to the point where a maximum yield is attained, then the plant yield decreases as the applied nutrient increases. In a study that explored the optimum Cu fertilizer for corn, Rodriguez and Ramirez (2005) reported that the relationship between the extracted Cu and the relative yield was quadratic. Results showed Cu to be both nutritional and toxic to *Brassica rapa*. Yield reduction could be due to poor root development in high Cu concentration. A similar observation has been reported for maize roots (Liu *et al*. 2001). As the Cu application rate increased, root growth

of maize decreased progressively. Roots fail to explore the soil for water and nutrients to be taken up into the plants due to the suppression effect of excess Cu on root elongation and branching restrictions (Wong and Bradshaw 1982).

Effects of Cu on Plant Height

Plant height relates to plant yield (*Fig. 2*). The highest plant height was observed at 10 mg Cu kg^{-1} in both Oxisol and Inceptisol. In Histosol, the highest plant height was recorded at 20 mg Cu kg^{-1}. Plants experienced more than 50% in height reduction at 15 mg Cu kg^{-1} compared to that of 10 mg Cu kg^{-1}, for both mineral soils. In Histosol, a Cu application rate ranging from 20 to 30 mg kg^{-1} reduced plant height by 50%. This concurs with Sonmez *et al.* (2006) who recorded a reduction in tomato plant height with increasing Cu application by 40.9 to 50.4% compared to the control treatment.

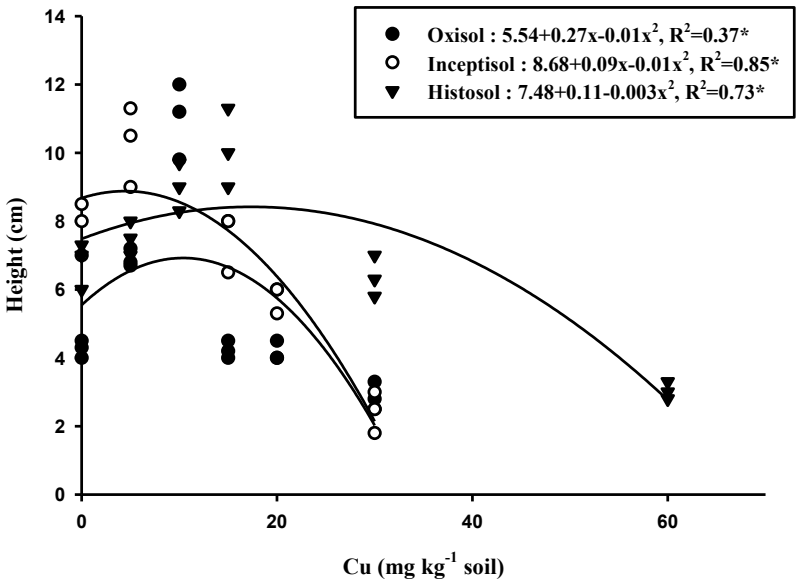


Fig. 2: Plant height response to increasing Cu concentrations in Oxisol, Inceptisol and Histosol

Effects of Cu on SPAD Value of Leaves

SPAD values correlate with the greenness or relative chlorophyll contents in leaves (Kariya *et al*. 1982). SPAD readings in plants grown on Oxisol and Inceptisol decreased drastically at 15 and 20 mg kg^{-1} Cu, respectively (*Fig. 3*). On the other hand, the SPAD values of plant grown on peat soil remained constant from 0 to 30 mg kg^{-1} Cu. No data were obtained from plants grown in Oxisol at 20 and 30 mg kg^{-1} Cu and Inceptisol at 30 mg kg^{-1} Cu due to small leaf size; moreover plants failed to thrive. Bernal *et al.* (2006) reported adverse effects of excess Cu such as the reduction in photosynthetic activity due to decreased content of photosynthetic pigments and the structure of chloroplasts. Specifically,

excess Cu decreases photosystem II activity and electron transfer rates (Janik *et al.* 2010). This could be explained by the decreasing SPAD value of leaves with increasing Cu application.

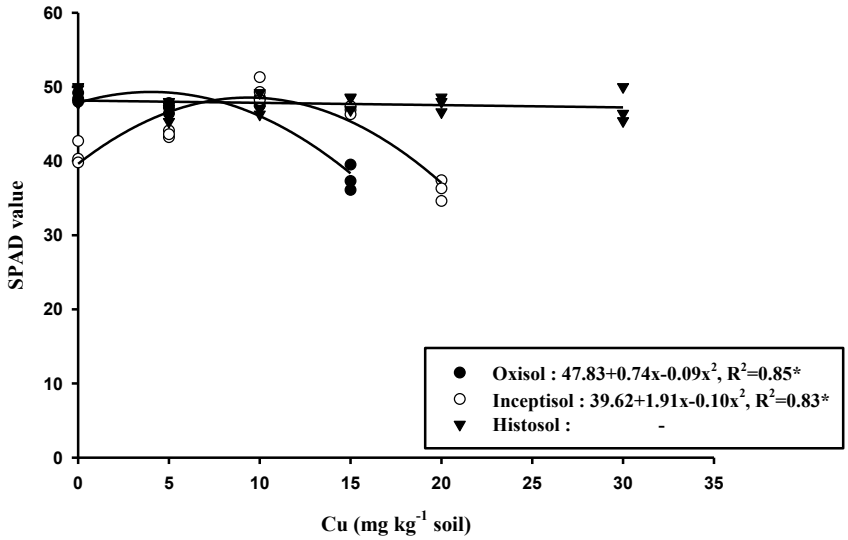


Fig. 3: SPAD values of leaves measured on plants grown in soils under increasing Cu concentrations

Fractionation of Cu in Oxisol and Inceptisol

All the six soil Cu fractions increased linearly with increasing Cu application rates. The relationships between each fraction with increasing Cu concentrations are shown in *Fig. 4*. Oxisol had a significantly higher amount of organic, Fe/Mn oxides and carbonate-bound Cu fractions than Inceptisols, at a rate of 60 mg Cu kg^{-1}. Both the Oxisol and Inceptisol were found to have the same order of Cu fractions; organic > residual > Fe/Mn oxides > carbonates > exchangeable > water soluble.

The water soluble and exchangeable fractions are the bioavailable Cu, readily available for plant and soil microbial uptake. These fractions were lower due to the high affinity of Cu to metal oxides, organic matter and carbonates (Sposito 2008). At the rate of 60 mg Cu kg^{-1}, Oxisol had higher carbonate-bound, Fe/Mn oxides-bound and organic fractions. This could be attributed to the application of ground magnesium limestone (GML) during soil preparation. Lime amendments increase soil pH but decrease Cu solubility as Cu becomes co-precipitated with carbonates. The presence of high content of Fe and Al hydroxides such as goethite (α-FeOOH), hematite (α-Fe_2O_3) and gibbsite (Kabata-Pendias 2001; Anda *et al.* 2008) is associated with Oxisol which had undergone advanced weathering. The particle size of aluminum hydroxides are extremely small and have highly reactive surfaces which increase Cu sorption in the soils. Goethite formation occurs in an oxic soil with a relatively high organic matter content, therefore, partly presenting

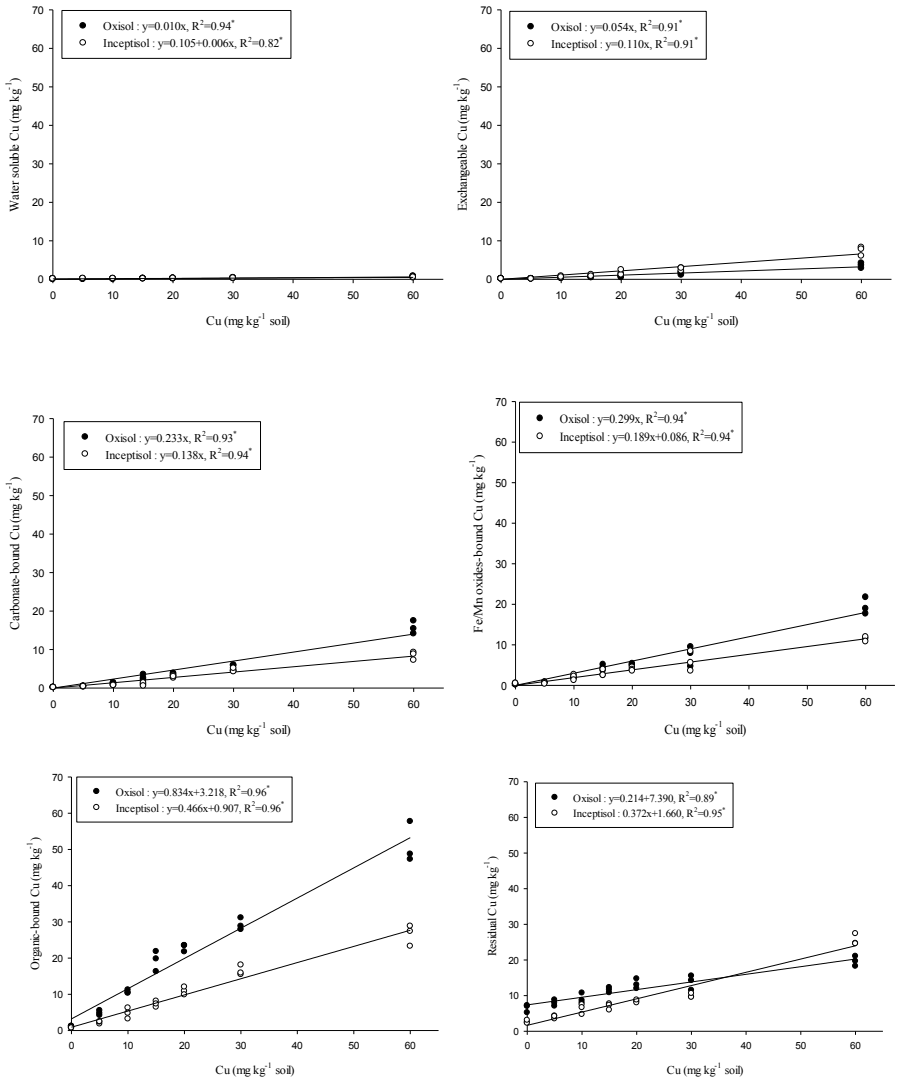


Fig. 4: Six operationally defined Cu fractions extracted from Oxisol and Inceptisol

as ternary complexes as it forms a coating on clay minerals and humic substances (Violante *et al*. 2005), while simultaneously increasing the Fe oxides-bound, organic and residual Cu fraction. The Oxisol contained higher (4.13%) soil organic matter content than Inceptisol (3.42%). This observation is similar to that of Lu *et al.* (2005) who reported that higher organic material content resulted in high Cu organic fraction. The Inceptisol showed a higher residual fraction than the Oxisol at 60 mg Cu kg^{-1}. The higher preference of Cu adsorption to organic matter is possible at higher copper concentrations, when the predominant clay mineral is kaolinite (Havlin *et al*. 1999), resulting in the reduction of residual Cu in Oxisol. Similarly, there is more resistant Cu fraction in the Inceptisol.

CONCLUSION

The application of Cu at low rates proved beneficial for *Brassica rapa* but was toxic at high rates as shown by the results of yield response, height and the SPAD values of leaves. The findings of this study suggest that the soil critical Cu level and toxicity threshold limits are influenced by the concentration of water soluble and exchangeable Cu. Copper distribution in Oxisol and Inceptisol is mainly controlled by the organic matter followed by clays, Fe and Al oxides. Soil organic matter content could be effective in reducing phytotoxicity of Cu at high levels of soil Cu concentration.

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