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ORIGINAL PAPER

Chemical fractions and phytoavailability of copper to rape grown in the polluted paddy soil

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Abstract This paper focuses on the phytoaccumulation and translocation of copper (Cu) in rape grown in the Cu-polluted paddy soil. Pot experiments were conducted in greenhouse conditions to examine the Cu availability and uptake by rape in a paddy soil. The soil was spiked with different concentrations of Cu (0, 100, 300, 500 and 1,000 mg kg^{-1} soil, added as $CuSO_4$) to simulate soil Cu contamination. After 8 months of growth, plant shoots, stems, pod shells and rapeseeds were harvested for analysis. The concentrations of Cu in the roots and aerial parts of the rape and available Cu in soils were then extracted and determined. Chemical fractions of Cu in the paddy soil of rape were also investigated by sequential extraction techniques. The findings showed that Cu in the clean paddy soil was mainly distributed in residual fractions. However, the most drastic increase was observed in Fe–Mn oxides-bound fractions and organic-bound fractions with increasing soil Cu concentrations. Exchangeable fractions played a more important role than other fractions in controlling the mobility and phytoavailability of Cu. Rape growth was stimulated by low concentrations of Cu, but inhibited by high concentrations. Compares to the aerial parts, the roots were more sensitive to Cu toxicity. The correlation analysis showed that Cu in exchangeable fractions made the greatest contribution on the accumulation of Cu in rapes. The factor analysis results showed that the exchangeable fractions in roots can be indicator of Cu availability. Meanwhile, the bio-concentration factors and the translocation factors of Cu in rape were determined and the results showed that Cu had lower accumulation in the edible parts of the rape.

Keywords *Brassica napus* L. · Chemical fractionation · Copper accumulation · Factor analysis · Phytoaccumulation

Introduction

Rape (*Brassica napus* L.) is one of the most important staple oil crops in a large part of the world, especially in China. Because of increased input from industry, traffic and agriculture, the average heavy metal content in soils has increased considerably (Salomons and Stigliani 1995). Heavy metal contamination in soil is a major problem for the environmental quality of the world (Yoon et al. 2006). Copper is an essential nutrient for plant growth at low concentrations, but excessive amounts are phytotoxic (Michaud et al. 2007).

The paddy soil is widely distributed in China, especially in the middle and lower reaches of Yangtze river, accounting for 1/5 of the cultivated land of China. In recent years, due to irrigation by wastewater coming from the industry and agriculture activities, some paddy soils have been polluted by heavy metal, containing Cu pollution. It will cause a serious threat to ecological environment and human health (Luo et al. 2003). In order to avoid the Cu pollution of the food chain, it is essential to assess Cu bioavailability in paddy soil. Furthermore, the bioavailability of Cu to rape in the paddy soil in middle and lower reaches of Yangtze River of China has not been reported. Therefore, there is significance to research the uptake of Cu by rape under Cu contamination.

In order to evaluate Cu activity in soil and to determine how readily Cu uptake by plants occur, it is essential to

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understand its mobility and bioavailability which depends on its chemical fractionations in the soil, rather than on the total amount accumulated (Zemberyova et al. 1998; Tao et al. 2003). In soil matrix, Cu can be associated with soil components and exist in a variety of chemical–physical forms including exchangeable fraction, Fe–Mn oxide-bound fraction, carbonate-bound fraction, organic-bound fraction and residual fraction in soils (Ahumada et al. 2009). There are different abilities to retain or release Cu in these fractions, as a result, it significantly influence the Cu mobility and bioavailability (Kabala and Singh 2001). Sequential extraction technique provides a powerful tool for evaluating metal forms (Tessier et al. 1979; Grzebisz et al. 1997). It has been used to estimate the distribution and potential bioavailability of Cu in soil (Maiz et al. 2000; Chaignon et al. 2003). Tessier's sequential extraction method has been widely used, in which heavy metals in soils were categorized in the five fractions: exchangeable fraction, carbonate-bound fraction, Fe–Mn oxide-bound fraction, organic-bound fraction and residual fraction (Tessier et al. 1979; Lucho-Constantino et al. 2005; Silveira et al. 2006; Rodríguez et al. 2009). Exchangeable fraction is bioavailable fractionation; carbonate-bound fraction, Fe–Mn oxide-bound fraction and organic-bound fraction are potential bioavailable fractionation; residual fraction is unbioavailable fractionation (Ma and Rao 1997; Rodríguez et al. 2009; Yang et al. 2011a).

Much work has been conducted to evaluate the availability, accumulation and translocation of Cu in the crops, such as rape, wheat and maize, to reduce Cu uptake by crops plants in Cu-polluted soils (Tao et al. 2003; Herrero et al. 2003; Chaignon et al. 2009; Guan et al. 2011). Previous studies have focused on plant physiological responses and Cu accumulation in Cu-contaminated soils (Johansson et al. 2005; Huang et al. 2009; Feigl et al. 2013). Other researches have showed that plant cultivation could increase the potentially available fraction of Cu in a polluted soil (Tao et al. 2003; Cattani et al. 2006). In addition, Brun et al. (2001) investigated the availability of Cu in Cu-contaminated vineyard soils and measured changes in the concentrations of Cu in the roots and aerial parts of the maize. Ali et al. (2011) also have researched on the remediation methods for arsenic removal from the ground water. However, further studies are still crucially important because the processes in soil are very complex and need more evaluation.

The factor analysis (FA) is a more adequate multivariable technique when the goal is not only to reduce the number of variables but also to detect structures in the relationships between variables (Maiz et al. 2000). In the present paper, we have tried to evaluate the metal availability from polluted soil to rape growing in greenhouse conditions. The total metal content in soil and the fractions obtained with the sequential extraction procedure, and the ones found using the Tessier method were considered in the analysis. FA was used to check the relationships between metal contents in rape and the different fractions in paddy soil.

The primary objective of this study was to investigate copper fractionation in the soil in an attempt to obtain a better understanding of its availability and subsequent uptake by rape. Meanwhile, the bioavailability and translocation of Cu in rape grown in the Cu-polluted paddy soil was also evaluated by a pot experiment in the current study. The research was carried out in Wuhu city within March 2006 to May 2007.

Materials and methods

Soil samples

The soil sample used in the study was collected from a paddy field of Qingshui in Wuhu city, Anhui province, China(118°48′E, 31°30′N). Soil samples were collected from 60 randomly selected sites in the paddy field at 0–20 cm depth. The main properties of soil are shown in Table 1. The total Cu concentration was in the range of the national environmental quality standard for agricultural soils (Cu 50 mg kg^{-1} in GB 15618-1995) issued by State Environmental Protection Agency of China.

Experimental design

The soil samples were air-dried and passed through a 2-mm sieve prior to the greenhouse pot experiment. Chemical fertilizers, at the rates equivalent to 0.3 g N, 0.2 g P and 0.3 g K kg^{-1} (dry weight) were applied as $(NH_4)_2SO_4$, KH_2PO_4 and K_2SO_4, were mixed with 5-kg air-dried soil (bulk soil) thoroughly in plastic pots (28 cm in height and 22 cm in diameter) before sowing. The experiment totally contained five treatments. The five treatments had been

Table 1 Physical and chemical properties of soil samples

Soil type	pH	EC μ cm^{-1}	Organic matter (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})	Total Cu (mg kg^{-1})	Total Zn (mg kg^{-1})
Paddy soil	7.32 ± 0.47	98 ± 9	17.22 ± 2.10	1.68 ± 0.17	0.34 ± 0.09	6.54 ± 0.85	32.80 ± 3.44	78.70 ± 5.61

spiked with a series of Cu^{2+} concentration (0, 100, 300, 500 and 1,000 mg kg^{-1} soil, added as $CuSO_4$), and watered and left to equilibrate outdoors for 2 weeks before planting vegetables. All the treatments were arranged in a complete random design with four replicates. Thirty seeds of rape named Huyou 16 (*Brassica napus* L.) were sowed in each pot, and the seedlings were thinned triple to the total of 10 in each pot. After cultivation for 45 days, the seedlings were thinned to the total of 3 in each pot. The moisture content of 60 % field water holding capacity was adjusted by weight during rape growth. Rape plants were harvested at mature stages (after 8 month) for analysis.

Soil and plant sampling

At the termination of the cultivation period, rape plants were harvested and soil samples were collected from the rhizosphere for fractionation. The soil samples were air-dried, ground and sieved to 2 mm for the analysis of available Cu, total Cu and Cu fractions. The plant samples were washed with running tap water and rinsed thoroughly with deionized water, dried at 70 °C for 24 h, and dry masses were recorded before copper determination. The dried plant samples were used for biomass determination, and then, they were stored in desiccators for total Cu analysis after being ground and sieved to 0.25 mm.

Analytical methods

The dried plant samples were digested by HNO_3–H_2SO_4–$HClO_4$ (8:1:1) after being triturated, and the concentrations of Cu were determined by atomic adsorption spectrophotometry (AA6800, Shimadzu, Japan); the Cu standard sample of the State Environmental Protection Agency of China was used for correction (Environmental Monitoring of China 1992). According to the sequential fractionation method, a proportion of liquid to soil of 10:1 was made for Cu fraction extraction. The sequential extraction method of Tessier et al. (1979) was used for the Cu fractions. Soil pH and the contents of water-soluble salts, organic matter and N, P, and K were measured by the methods of Environmental Monitoring of China (1992). Soil samples were extracted by 0.1 mol L^{-1} HCl (liquid:soil = 5:1) for 90 min, to determine the concentration of available Cu in soils. The content of Cu was determined by atomic adsorption spectrophotometer (AAS) [AA6800, Shimadzu, Japan]. All chemicals were of analytical grade, and all plastic and glassware were washed before use, soaked in 2 % HNO_3 for more than 24 h, and rinsed with deionized water.

Statistical analysis

All results were reported as the mean of four replicates, and all data were subjected to one-way analysis of variance with SPSS 13.0 statistics software. Regression, correlation analysis and *t* test were used to determine the significance of difference among various groups of plant and soil samples.

Results and discussion

Chemical fractions distribution of Cu in soil

Figure 1 shows that Cu chemical fractions contents all increased with addition of any Cu treatment in soil. With increasing Cu concentrations in paddy soils, the concentrations of Cu in Fe–Mn oxides-bound fractions, organic-bound fractions and residual fractions increased greatly, relative to the control, while the exchangeable fractions and carbonate-bound fractions in soils increased indistinctively in 0 mg kg^{-1} treatment and 100 mg kg^{-1} treatment. With the growth of rape in soils, the concentrations of Cu in exchangeable fraction was more than carbonate-bound fraction in 500 mg kg^{-1} treatment and 1,000 mg kg^{-1} treatment, whereas the relationship was reversed for lower concentration treatment groups. The parameter fractionation distribution coefficient (FDC) was usually defined as the one metal fractionation content accounting for the percentage by total amount of the same

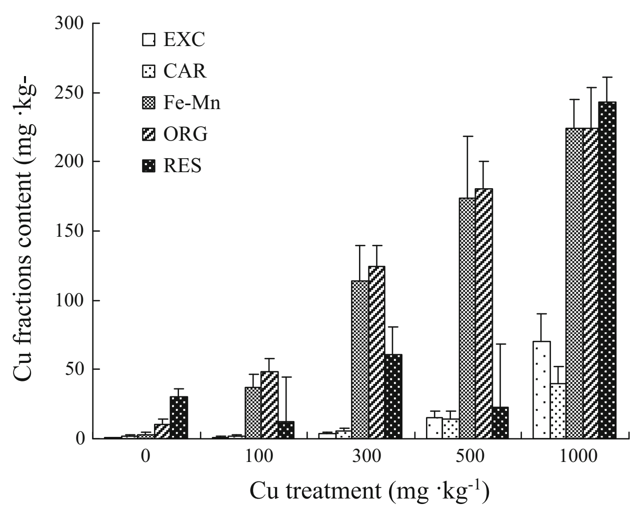

Fig. 1 Distribution of Cu fractions in the paddy soil in which rape was grown for 8 month. *EXC* the exchangeable fractions, *CAR* the fraction associated with carbonates, *Fe–Mn* Fe–Mn oxides-bound fractions, *ORG* the fraction associated with the organic matter and/or sulfides, *RES* the residual fraction

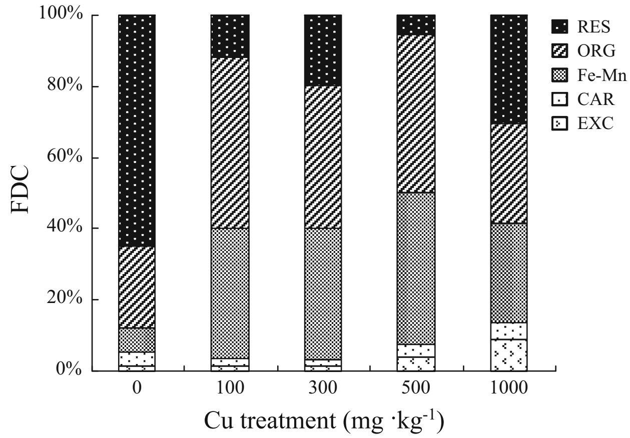

Fig. 2 Fraction distribution coefficient (FDC) of Cu in the paddy soil in which rape was grown for 8 month. Values were means of four replicates. *EXC* the exchangeable fractions, *CAR* the fraction associated with carbonates, *Fe–Mn* Fe–Mn oxides-bound fractions, *ORG* the fraction associated with the organic matter and/or sulfides, *RES* the residual fraction

metal. The FDCs of Cu increased significantly in Fe–Mn oxides-bound and organic-bound ($P < 0.001$) with increasing soil Cu concentration (0–500 mg kg^{-1}) probably because of greater mobility of Cu in the paddy soil. However, FDCs of Cu in Fe–Mn oxides-bound and organic-bound decreased partly, when soil Cu concentration reached 1,000 mg kg^{-1}. FDC of Cu reduced significantly in residual fraction ($P < 0.001$), in range of 0–500 mg kg^{-1}. However, FDC of residual fraction increased obviously when soil Cu concentration reached 1,000 mg kg^{-1}, compared to 100, 300 and 500 mg kg^{-1} treatment. FDCs of Cu in exchangeable fraction and carbonate-bound fraction did not rise significantly until soil Cu concentration reached 500 and 1,000 mg kg^{-1} (Fig. 2).

In the sequential extraction procedure, exchangeable, carbonate-bound, Fe–Mn oxides-bound, organic-bound and residual Cu were partitioned, respectively. Previous studies showed that in the clean soil, residual fractions was mainly heavy metals fraction, which was unbioavailable for the plants (Guo and Zhou 2005; Yang et al. 2011b), but the percentage of heavy metals associated with available fraction increased with increasing total amount of heavy metals (Guo and Zhou 2005). It was in agreement with the results obtained in present study. In the present study, Cu was primarily accumulated in the residual fractions (64.72 %) and organic-bound fractions (23.02 %) in bulk soil (the control), indicated that Cu had stronger ability to associate with the crystalline structures of the minerals and the organic ligand (Fuentes et al. 2004; Guan et al. 2011). The results showed that residual fractions were mainly Cu fraction in unpolluted paddy soil, however, with the increase in amounts of Cu in the soil, bioavailable fractions increased significantly. A series of interactions (e.g., adsorption, precipitation, complexation, etc.) would take place with the extraneous Cu addition. Cu was primarily adsorbed to the strongest binding sites such as soil organic matter and accumulated in organic-bound fractions (Cao and Hu 2000; Marcato et al. 2009; Guan et al. 2011). The Cu in soil was firstly precipitated at the stronger competitive sites as Fe and Mn oxides and then at weaker sites such as carbonates (Cao and Hu 2000). Therefore, the Fe–Mn oxides-bound fractions increased most drastically with increased soil Cu concentrations. Cu chemical fractions in soil were dependent on Cu addition. When Cu addition was higher than 100 mg kg^{-1}, the residual fractions significantly declined but the Fe–Mn oxides-bound and organic-bound fractions increased markedly. The results suggested that the cultivation of the rape in paddy soil can induce the transformation of bound Cu from less mobile fractions to bioavailable fractions.

The biomass of rape tissues and the Cu uptake

The biomass of rape tissues

The change tendency of plant height and root length and fresh weight of rapes roots, stems, pod and rapeseeds under Cu treatments are shown in Table 2. The plant height of rape increased to a certain extent (86.30 cm at 100 mg kg^{-1} Cu addition) and then significantly decreased with the increased Cu addition (Table 2). The addition of Cu obviously inhibited the root elongation of rape in paddy soils, compared to the control (0 mg kg^{-1}). Table 2 also shows the fresh biomass of rape roots, stems, pods and rapeseeds under Cu treatments. The fresh biomass of rape stems increased to a certain extent (9.76 g at 100 mg kg^{-1} Cu addition) and then significantly decreased with the increased Cu addition, it indicated that this value (100 mg kg^{-1} Cu addition) was hazardous threshold of rape. The fresh biomass of roots and pods decreased significantly with the increased Cu addition. However, the fresh biomass of rapeseeds increased till the Cu addition at 300 mg kg^{-1} and the magnitude was 0.17 g. Afterward, the fresh biomass decreased significantly to 2.40 g for stems and 0.07 g for rapeseeds when Cu^{2+} was added at 1,000 mg kg^{-1}. These results indicated that the toxic effect of Cu on rapes had threshold values. Rapes growth could be stimulated by low concentrations of Cu ($\leq$100 mg kg^{-1}), but inhibited by high concentrations (1,000 mg kg^{-1}). The results were consistent with reports that heavy metals influence root tip cell mitosis, decrease the rate of cell division, and cause biomass to decrease (Yang et al. 2002; Seregin and Kozhevnikova 2006).

Table 2 Effects of Cu on the growth and matter accumulation of rape in different treatments

Treatment (mg kg^{-1})	Plant height (cm)	Root length (cm)	Fresh weight				Pod number
			Stem (g $plant^{-1}$)	Root (g $plant^{-1}$)	Pod (g $plant^{-1}$)	Rapeseeds (g pod^{-1})	
0	80.70 ± 2.30	13.00 ± 1.80	7.63 ± 1.12	5.87 ± 0.59	45.10 ± 2.74	0.15 ± 0.03	82 ± 6
100	86.30 ± 3.50	8.20 ± 3.10	9.76 ± 0.75	4.79 ± 0.82	27.61 ± 2.19	0.16 ± 0.04	78 ± 4
300	78 ± 2.70	10.00 ± 1.10	4.66 ± 0.78	2.26 ± 0.92	20.12 ± 1.72	0.17 ± 0.09	39 ± 5
500	67.40 ± 2.50	9.20 ± 0.96	6.83 ± 0.53	3.22 ± 0.43	14.57 ± 0.77	0.12 ± 0.12	47 ± 6
1,000	45.90 ± 1.90	8.70 ± 0.78	2.40 ± 0.32	2.60 ± 0.09	6.91 ± 0.59	0.07 ± 0.08	31 ± 3

Values are mean ± standard deviation (SD) of four replication samples

Table 3 Cu concentrations in rape tissues in different treatments and the transfer ratios in rape-soil system

Treatment (mg kg^{-1})	Cu concentrations in rape tissues (mg kg^{-1}) and the transfer ratios								
	Roots	BCF_{root}	Stems	BCF_{stem}	$TF_{Stem/root}$	Pods shell	Pods shell/stem	Rapeseeds	Rapeseeds/pods shell
0	26.70 ± 0.74	0.57	25.35 ± 0.59	0.54	0.95	16.40 ± 1.26	0.65	8.10 ± 0.77	0.49
100	74 ± 4.20	0.73	14.85 ± 9.58	0.15	0.20	15.20 ± 4.41	1.02	8.28 ± 1.38	0.54
300	115.65 ± 8.49	0.37	24.85 ± 7.27	0.08	0.21	40.55 ± 5.97	1.63	14.13 ± 0.77	0.35
500	267.85 ± 11.95	0.66	10.80 ± 6.88	0.03	0.04	47.85 ± 6.17	4.43	14.55 ± 0.47	0.30
1,000	1001.65 ± 53.20	1.25	20.10 ± 5.73	0.03	0.02	21.3 ± 3.79	1.06	12.18 ± 1.53	0.57

Values are mean ± standard deviation (SD) of four replication samples

Accumulation of Cu in rape tissues

The amounts and distribution of Cu accumulated in rapes under different treatments are shown in Table 3. The Cu concentrations in roots increased significantly with increased soil Cu concentrations and the magnitude ranged from 26.70 to 1001.65 mg kg^{-1} (Table 3). However, in general, compared with the control, rape stems' Cu concentrations did not decrease significantly, with the increased soil Cu concentrations. The Cu concentrations in pods shell and rapeseeds increased by 2.92-folds and 1.80-folds, respectively, at the Cu level of 500 mg kg^{-1}. Afterward, the Cu concentrations decreased significantly to 21.30 mg kg^{-1} for pods shell and 12.18 mg kg^{-1} for rapeseeds, when Cu^{2+} was added at 1,000 mg kg^{-1}.

Bio-concentration factor (BCF) was calculated as the ratio of Cu concentration in rape roots or aerial parts to the total concentration in soil. Table 3 shows BCF_{root} was more than 1.0 in 1,000 mg kg^{-1} treatment. As the time of rape growth, a number of Cu^{2+} entered into the tissues of rape roots. $TF_{stem/root}$ gradually decreased with increased soil Cu concentrations. When the soil Cu concentration reached 1,000 mg kg^{-1}, BCF_{stem} reached the minimum value (0.03), decreased by 17-folds, comparing with the control. Rape (*Brassica napus* L.) showed a ability for preventing Cu^{2+} to enter into the aerial parts. The observed trend of changes in BCF_{stem} in different treatments also paralleled the changes in $TF_{Stems/roots}$ (Table 3).

Different from roots, the concentrations of Cu in rape stems were in a narrow range. The values varied from 25.35 to 20.10 mg kg^{-1}, but were not significantly influenced by Cu addition. The ratio of stems/roots was 0.95 in the least Cu-containing soils (0 mg kg^{-1}), but it declined to 0.02 after 1,000 mg kg^{-1} Cu addition. When the soil Cu concentration reached 500 mg kg^{-1}, the ratio of pods shell/stems reached the maximum value (4.43), increased by 6.82-folds, comparing with the control. But the ratio of pods shell/stems was reduced obviously with the concentration of Cu increasing. Unlike the ratio of pods shell/stems, the ratio of rapeseeds/pods shell was in a narrow range. The values varied from 0.49 to 0.57, but were not significantly influenced by Cu addition.

The BCF_{root} of Cu can imply the capability of Cu entry from soil to plant, and the values were less than 1.00 (in range of 0–500 mg kg^{-1} Cu addition), suggesting that only a small portion of Cu was transferred into the rape while large amount was still accumulated in soil. It indicated that just a small amount of copper had transferred into the aerial parts, the majority of copper had retained in root. That is to say, rape appeared to be capable of limiting Cu

Table 4 Correlation coefficient (*R*) between chemical fractions of Cu in the paddy soil and concentrations of Cu in rape root, stem, pod and rapeseed

Cu fractions	Root	Stem	Pod shell	Rapeseed
EXC	0.997** ($P < 0.001$)	−0.061 ($P = 0.923$)	−0.092 ($P = 0.884$)	0.281 ($P = 0.647$)
CAR	0.988** ($P = 0.002$)	−0.123 ($P = 0.844$)	0.031 ($P = 0.961$)	0.389 ($P = 0.517$)
Fe–Mn	0.804 ($P = 0.101$)	−0.322 ($P = 0.597$)	0.497 ($P = 0.394$)	0.772 ($P = 0.126$)
ORG	0.786 ($P = 0.115$)	−0.333 ($P = 0.583$)	0.519 ($P = 0.370$)	0.789 ($P = 0.113$)
RES	0.941* ($P = 0.017$)	0.211 ($P = 0.733$)	−0.177 ($P = 0.776$)	0.226 ($P = 0.715$)

* Significant at level $P < 0.05$, ** significant at level $P < 0.01$

translocation from soil to roots to avoid excessive Cu-induced damage. The reason may be due to Cu fractions in the rhizosphere soil influenced by rape growth time. This resistance capacity may be attributed to the complexing agents from roots exudates could form stable complexes with Cu to decrease Cu bioavailability (Ahumada et al. 2009; Torri and Lavado 2009), and the root/mycorrhizal structures of rape could potentially limit the Cu uptake into the symplasm (Zhang et al. 2009). In addition, root-induced changes in dissolved organic carbon, redox potential and microbial activity in the rhizosphere may cause the change in copper fractionation (Tao et al. 2003), limiting the movement of Cu. In line with some former investigations, Cu was found to accumulate more in the roots than in the aerial parts (Lexmond 1980; Brun et al. 2001; Chaignon et al. 2003; Ahumada et al. 2009; Guan et al. 2011). This has been described as a mechanism of plant tolerance to metal toxicity. The accumulation of metals in roots would minimize the adverse effects of metals on shoot growth (Chaignon et al. 2009); thus, metal sequestration in roots could be considered as a major adaptation of plants to metal stress (Clemens et al. 2002).

If the load of Cu was extremely high, e.g., greater than 500 mg kg^{-1}, the resistance of roots to Cu declined drastically and the Cu accumulation in roots significantly increased. It was in agreement with the results obtained in present study where the BCF_{root} of Cu was more than 1.0, when soil Cu concentrations reached 1,000 mg kg^{-1} (Table 3). As shown in Table 3, the gradual decline of stems/roots ratios with increased Cu suggested that an important restriction occurred in the internal transport from roots to stems. The main enrichments of Cu in roots were evident in many cases, especially at higher Cu loading (Bhattacharyya et al. 2006; Ahumada et al. 2009; Guan et al. 2011). However, the resistant effect was limited when extremely large quantities of Cu were added. The rapeseeds/pod shells ratios showed the capability of Cu entry from pod shells to rapeseeds, and the values were less than 1.00 in all Cu treatments, suggesting that rape appeared to be capable of restricting Cu translocation from pod shells to rapeseeds to avoid excessive Cu-induced damage.

Table 5 Linear regression analysis between the available Cu content in soil and Cu contents in rape root, stem, pod shell and rapeseed

Regression equation	*R* value	Significance
$Y_{root} = 1.418X_{available\ Cu} - 71.150$	0.968	0.007
$Y_{stem} = -0.003X_{available\ Cu} + 19.933$	0.125	0.841
$Y_{pod\ shell} = 0.01X_{available\ Cu} + 25.782$	0.176	0.777
$Y_{seed} = 0.006X_{available\ Cu} + 9.880$	0.535	0.353

Y means Cu contents in rape root, stem, pod shell and seed; X means available Cu content in soil

Relationships among Cu availability, Cu chemical fractions in soil and the uptake by rape

The correlation analyses were performed among the Cu concentrations in rape tissues, available Cu and different chemical fractions of Cu in soil. As shown in Table 4, the Cu concentrations in roots presented significant correlation relationship with the EXC-Cu and CAR-Cu in soil ($R > 0.90$, $P < 0.01$), respectively. Similarly, the Cu concentrations in roots also presented significant correlation relationship with the RES-Cu in soil ($R > 0.90$, $P < 0.05$). However, no significant correlations were found between EXC-Cu and Cu concentrations among stems, pod shells and rapeseeds, except roots (Table 4).

Linear regression models were perfectly fitted between the Cu concentrations in rape tissues (as dependent variable) and Cu available fractions in soil (as independent variables). Table 5 shows the Cu available fractions were significantly related with the Cu in roots with *R* higher than 0.90 ($P < 0.01$). The Cu concentrations in stems, pod shells and seeds exhibited weaker relations with the concentrations of Cu available fractions for *R* was 0.125, 0.176 and 0.535, respectively.

Relationships between the available Cu and fresh biomass of rape in different treatments

Table 6 shows linear regression models between the available Cu concentrations in soils (as independent variables) and biomass of rape in roots, stems, pod shells and rapeseeds (as dependent variable). The Cu available fractions were significantly related with the biomass of rapeseeds for R was 0.895 ($P < 0.05$). All the coefficients in front of the available Cu content were negative. The biomass of roots, stems and pod shells exhibited weaker relations with the concentrations of Cu available fractions for R was 0.719, 0.857 and 0.864 ($P > 0.05$), respectively.

The observed results in our research are in agreement with the earlier reports that Cu in roots had relatively good sensitivity to Cu pollution, while the aerial parts of rape may not directly reflect the phytotoxicity of Cu (Guan et al. 2011). Meanwhile, the results also suggested that Cu in exchangeable fractions made the greatest contributions on the accumulation of Cu in the roots of rape (Table 4). In the case of the paddy soils studied, the lack of correlation found for rape between Cu concentrations in roots and in the aerial parts, suggests that an analysis of the aerial parts would not be a good indicator of plant Cu uptake.

The linear regression analysis between the available Cu content in soil and Cu contents in root, stem, pod shell and rapeseed of rape showed that available Cu made the largest contribution on the accumulation of Cu in roots. The exchangeable fractions and available Cu played a more important positive role in Cu bioavailability due to the biggest positive number of its coefficient, thus was more associated to estimate the actual toxicity of Cu to plant in our case. It could be presumed that Cu in plants mostly came from direct absorption of available fractions in the soil. The contents of available fractions of Cu were higher than the sum of the exchangeable fractions, carbonate-bound fractions and Fe–Mn oxides-bound fractions, but lower than that of the first four fractions (data not shown), suggesting that available fractions of Cu could extract exchangeable, carbonate-bound, Fe–Mn oxides-bound and a portion of stable organic-bound fractions. Compared to the residual fraction, carbonate-bound fraction and Fe–Mn oxides-bound fraction and organic-bound fraction had a high potential to become bioavailable fraction, and therefore, they served as a potential nutrition pool to plants. If the proportions of these fractions changed, the capacity and mobility of heavy metals in soil would change (Zhou and Sun 2002; Zhou et al. 2004). In fact, the available fractions of Cu in soil are highly mobile. As the weakly bound fractions, such as carbonate-bound fraction and Fe–Mn oxides-bound fraction, tend to resolve into available fractions. Therefore, the importance of these fractions should not be neglected.

Factor analysis

The factor analysis technique allows a considerable reduction in the number of variables and the detection of structure in the relationships, which would give information about the relation between soil and plant systems (Maiz et al. 2000). In the present study, factor analysis (FA) was performed by evaluation of principal components and computing the eigenvectors. Table 7 presents the eigenvalues, the percentage of variance and the cumulative percentage of variance associated with each other. The results revealed that the first two factors explain approximately 91.501 % of total variance. Table 8 shows the loading of varimax rotated factor matrix for two-factor model. This is to be expected because these factors are extracted successively, each one accounting for as much of the remaining variance as possible. Table 8 displays the varimax rotated factor scores. Plots of factor loadings are shown in Fig. 3. The first and second factors explained 91.501 % of the total variation. The first factor, explaining 70.405 % of the total variation, exhibited a high positive factor loading on EXC and ROOT. The second factor, explaining 21.096 % of the total variation, exhibited a high positive factor loading on SHELL and SEED. It indicated that the capacity of factor 1 was higher than factor 2 in explaining the total variation. The results of factor analysis suggested that exchangeable fractions contents (EXC), Cu contents in roots (ROOT), shells (SHELL) and seeds (SEED) had relatively good sensitivity to the Cu pollution. As the critical variables of factor 1, the exchangeable fraction contents (EXC) and Cu contents in roots (ROOT)

Table 6 Linear regression analysis between the available Cu content in soil and biomass of rape root, stem, pod shell and rapeseed

Regression equation	R value	Significance
$Y_{root} = -0.004X_{available\ Cu} + 4.785$	0.719	0.171
$Y_{stem} = -0.009X_{available\ Cu} + 8.533$	0.857	0.063
$Y_{pod\ shell} = -0.046X_{available\ Cu} + 34.702$	0.864	0.059
$Y_{seed} = -0.00013X_{available\ Cu} + 0.167$	0.895	0.040

Table 7 Eigenvalues, percent of variance, cumulative percent of variance for the factor analysis of Cu content in soil and biomass of rape root, stem, pod shell and rapeseed

Factor	Eigenvalue	Variance (%)	Cumulative (%)
1	7.745	70.405	70.405
2	2.321	21.096	91.501
3	0.913	8.298	99.799
4	0.022	0.201	100.000

Table 8 Loading for varimax rotated factor matrix of two-factor model explaining 91.5 % of the total variance

Variable	Factor 1	Factor 2
EXC	0.996	−0.011
CAR	0.988	0.112
Fe–Mn	0.817	0.577
ORG	0.799	0.600
RES	0.975	−0.149
TOTAL	0.950	0.311
AVAILABLE	0.968	0.248
ROOT	0.996	0.019
STEM	−0.001	−0.525
SHELL	−0.067	0.968
SEED	0.318	0.898

EXC exchangeable fractions, *CAR* carbonate-bound fractions, *Fe–Mn* Fe–Mn oxides-bound fractions, *ORG* organic-bound fractions, *RES* residual fractions, *TOTAL* total Cu content in soils, *AVAILABLE* available Cu, *ROOT* Cu content in roots, *STEM* Cu content in stems, *SHELL* Cu content in pod shells, *SEED* Cu content in seeds

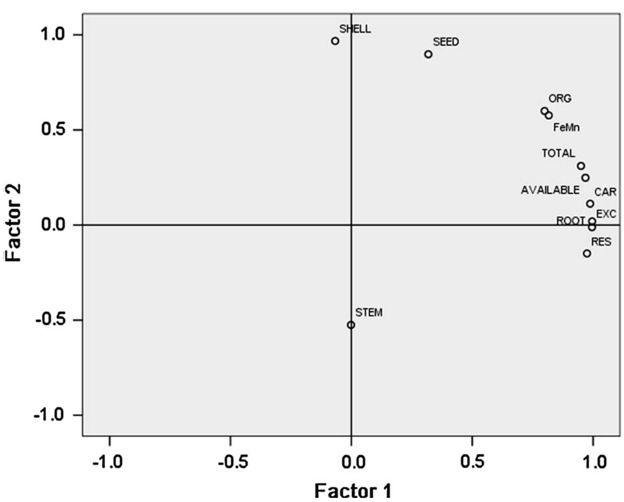

Fig. 3 Plots of variables on the first two axes extracted by factor analysis (with varimax rotation) of the data for different treatments

could be regarded as the best indicators for estimating the actual toxicity of Cu to rape. From Fig. 3, the variates loading heavily on axis I in the data analysis are those clearly associated with fractions contents of Cu in soils and Cu content in roots (EXC, CAR, RES and ROOT), while the variates loading heavily on axis II in this analysis are all related to Cu content in aerial parts (STEM and SHELL).

Conclusion

The present study demonstrated that the Cu mobility and its availability to the rape were highly dependent on the chemical fractions in the polluted paddy soils. The residual fractions accounted for the largest proportion of Cu in the unpolluted paddy soil; however, the Fe–Mn oxides-bound fractions and organic-bound fractions increased most rapidly with the increase of Cu addition. The exchangeable fractions played the most important role in controlling the Cu mobility and availability, which mainly associated to the Cu uptake by rape.

Low concentrations of Cu facilitated the growth of rape, but high concentrations of Cu obviously inhibited the growth of rape. Comparing with the accumulation in soil, uptake of Cu in rape was very low. There is an important restriction occurred in the internal transport of Cu from roots to aerial parts. The exchangeable fractions made the most contributions on Cu uptake by rape roots. The factor analysis results showed that the exchangeable fractions and Cu contents in roots could be regarded as the best indicators for estimating the actual toxicity of Cu to rape.

Bio-concentration factors of Cu in roots were greater than those in stems. Translocation factors of Cu in stems were all less than 1.00 in all treatments, and the ratios of rapeseeds/pods shell were less than 1.00. The translocation of Cu was restricted from pod shells to rapeseeds to avoid excessive Cu-induced damage. Copper mainly accumulated in the underground parts of rape in our case.

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Abbreviations

BCF	Bio-concentration factor
CAR	Carbonate-bound fractions
Cu	Copper
EXC	Exchangeable fractions
FA	Factor analysis
FDC	Fractionation distribution coefficient
Fe–Mn	Fe–Mn oxides-bound fractions
ORG	Organic-bound fractions
RES	Residual fractions
TF	Translocation factor

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