Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 21(2009) 647–653
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Combined toxicity of copper and cadmium to six rice genotypes (*Oryza sativa* L.)

HUANG Yizong*, HU Ying, LIU Yunxia

Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China. E-mail: hyz@rcees.ac.cn

Received 22 May 2008; revised 08 August 2008; accepted 01 September 2008

Abstract

Accumulations of copper (Cu) and cadmium (Cd) in six rice cultivars (94D-22, 94D-54, 94D-64, Gui630, YY-1, and KY1360) were evaluated through exposure to heavy metal contamination (100 mg/kg Cu, 1.0 mg/kg Cd, and 100 mg/kg Cu + 1.0 mg/kg Cd) in a greenhouse. The dry weights of shoot and root, concentrations of Cu and Cd in plant tissues and the Cu, Cd, P, Fe concentrations in the root surface iron plaques were analyzed eight weeks later after treatment. The results indicated that the plant biomass was mainly determined by rice genotypes, not Cu and Cd content in soil. Separated treatment with Cu/Cd increased each metal level in shoot, root and iron plaques. Soil Cu enhanced Cd accumulation in tissues. In contrast, Cu concentrations in shoot and root was unaffected by soil Cd. Compared to single metal contamination, combined treatment increased Cd content by 110.6%, 77.0%, and 45.2% in shoot, and by 112.7%, 51.2% and 18.4% in root for Gui630, YY-1, and KY1360, respectively. The content level of Cu or Cd in root surface iron plaques was not affected by their soil content. Cu promoted Fe accumulation in iron plaques, while Cd has no effect on P and Fe accumulation in it. The translocation of Cu and Cd from iron plaques to root and shoot was also discussed. These results might be beneficial in selecting cultivars with low heavy metal accumulation and designing strategies for soil bioremediation.

Key words: Cu; Cd; combined contamination; soil; uptake; accumulation; root surface iron plaques

DOI: 10.1016/S1001-0742(08)62320-7

Introduction

To meet the needs of the rapidly increasing population, many countries have expanded tremendously the development of land and mineral resources. The emergence of many refinery factories has caused serious environmental problems including soil heavy metal contamination. The most concerned heavy metals are Cd, Pb, As, Hg, Cu, Cr, etc. According to the US National Priority List (NPL), for 1200 soil samples, 63% of them contain Pb, Cr, Cd and Cu at 15%, 11%, 8% and 7%, respectively (Hazardous Waste Consultant, 1996). In China, Cd, As, Pb, Hg and Zn have contaminated about 2.0×10^7 hm^2 which comprises 1/5 of the arable land. The industrial wastewater irrigation accounts for 3.3×10^7 hm^2 (Chen, 1996). Heavy metals in the soil can subsequently enter food chain and are considered hazardous to human health.

Cd is a non-essential element and can be absorbed easily by plants. Therefore, it is more toxic to plants than other metals such as Ni, Cu, Zn and Pb (Balsberg, 1989). Cu is an essential element, but the overdose can adversely affect plant growth (Cao *et al*., 2000; Chatterjee and Chatterjee, 2000).

The knowledge of heavy metals biological toxicity on plant, especially on growth and development, their uptake and accumulation in plants and their translocation dynamics, is very important for agricultural production and environmental and human health protection. Extensive research works have been performed in the related field. However, previous work is mostly about single metal contamination (Das *et al*., 1997; Tang and Robson, 2000; Sarret *et al*., 2002; Carrier *et al*., 2003; Murakami *et al*., 2007). The real situation is that soil is often contaminated with several heavy metals. Therefore, the increasing attention have been paid on the combined contamination (Stewart and Malley, 1999; Franklin *et al*., 2002; Wang, 2003; Ali *et al*., 2004; Alexander *et al*., 2006).

Response to soil heavy metal contamination is depended on the species, or even genotypes-specific. For example, compared to spring wheat, barley, corn and oats, the hard wheat, sunflower and flax are considered high Cd accumulation crops (Grant *et al*., 1999). Difference in heavy metal uptake and accumulation among cultivars are observed in soybean (Boggess *et al*., 1978), corn (Florijn and van Beusichem, 1993), wheat (Oliver *et al*., 1994; Zhang *et al*., 2000), barley (Wu and Zhang, 2002), potato (McLaughlin *et al*., 1994) and lettuce (John and Laerhoven, 1976; Costa and Morel, 1994). The Cd accumulation in crops varied with genotypic difference has also been reported by Grant *et al*. (1999). Moreover, Liu *et al*. (2006) investigated arsenic sequestration in iron plaque and its accumulation

* Corresponding author. E-mail: hyz@rcees.ac.cn

jesc.ac.cn

and speciation in different rice cultivars (94D-22, 94D-54, 94D-64, Gui630, YY-1, and KY1360).

Rice is one major grains crop, and 90% of production occur in Asia. Although soil heavy metal contamination can seriously affect the yield quantitatively and qualitatively, very few studies have been conducted on characterizing different rice cultivars grown on contaminated soil. The current research is expected to provide information for selecting rice cultivars which has low copper and cadmium accumulation capacity.

1 Materials and methods

1.1 Soil preparation

Soil samples were collected from paddy field at 0–20 cm depth in the vicinity of Huzhou, Zhejiang Province, China. The soil was air-dried, ground into fine powder and passed through a 1-mm sieve for growth experiment in the future. The physiological and chemical properties of soil were determined according to the methods described by Lu (1999). Soil pH is 6.3 determined from the water extracts (soil:water, 1:2.5, *W/W*). Organic matter content is 43.6 g/kg. Cation exchange capacity (CEC) is 11.2 cmol/kg, and Cu and Cd content are 40.0 and 0.5 mg/kg, respectively. Soil particles comprise 11.6% clay, 38.0% silty particle and 50.4% sandy particle.

1.2 Plant culture

Six rice (*Oryza sativa* L.) genotypic cultivars of 94D-22, 94D-54, 94D-64, Gui630, YY-1, and KY1360 were provided by Prof. Li Damo in the Subtropical Agricultural Ecological Institute of Chinese Academy of Sciences. Surface sterilization was conducted by soaking the seeds in 10% H_2O_2 for 10 min, followed by washing with deionized water three times. Germinated seeds were grown in perlite for 2–3 week until 3–4 leaf stage. The seedling growth conditions are 28°C/20°C (day/night), 14 h/10 h photoperiod with light intensity at 260–350 mmol/(m^2·s) and 60%–70% relative humidity.

For heavy metal application, stock solutions of $CuSO_4$ and $CdCl_2$ were mixed with 1000 g soil at the following four levels: (1) 0 (CK); (2) 100 mg Cu as $CuSO_4$ (Cu); (3) 1.0 mg Cd as $CdCl_2$ (Cd); (4) 100 mg Cu as $CuSO_4$ + 1.0 mg Cd as $CdCl_2$ (Cu + Cd). Cu and Cd were dissolved in water and mixed into soil and then wetted with deinonized water for one month to let reach equilibrium before used as potting mixture. Each Polyvinyl Chloride (PVC) pot (7 cm width × 25 cm height) was filled with 1000 g soil and planted with a single seedling, and fertilized with urea (0.428 g/kg) and potassium sulphate (0.247 g/kg). Each treatment was repeated 4 times and totally was 96 pots. Deionized water was applied every two days to maintain waterlogging condition. The plants were grown for 8 weeks at 25–30°C and light intensity was 500–1100 mmol/(m^2·s).

1.3 Plant tissue analysis

After the harvest, the rice root surface iron plaques were extracted with dithionite-citrate-bicarbonate (DCB) (Meharg and Jardine, 2003). Briefly, roots were rinsed with deionized water before soaked in a 40-mL solution consisting 0.03 mol/L sodium citric ($Na_3C_6H_5O_7·2H_2O$) and 0.125 mol/L sodium carbonate ($NaHCO_3$) for 10 min, and then continued for additional 1 h after adding 1 g $Na_2S_2O_4$. Eventually, root was washed several times with deionized water and the collected liquid was diluted to 100 mL. The solution was filtered and stored until analysis. Dry weight (dw) of root and shoot was determined after oven drying at 70°C for 72 h.

Plant samples were all digested prior to mineral analysis. The shoot and root tissues were grounded into fine power. A 0.25-g of sample was put into digestion tube and then 5 mL nitric acid (HNO_3) was added. After boiling at 90°C for 1 h, the temperature was raised to 160°C and all samples were melted. The digestion solution was added to 50 mL with ultrapure water. Calibration was conducted using contaminated tea leaf (GBW 07605(GSV-4) provided by the China National Standard Material Center to ensure that the recollection ratio reached 95%. Cu, Cd, Fe and P concentrations were determined using ICP-MS (Agilent 7500i, USA).

1.4 Data analysis

Iron plaque-to-shoot transfer factors (F_{shoot}) and iron plaque-to-root transfer factors (F_{root}) were calculated as follows:

$$F_{shoot} = \frac{C_{shoot,dry}}{C_{iron,plaque}} \tag{1}$$

$$F_{root} = \frac{C_{root,dry}}{C_{iron,plaque}} \tag{2}$$

where, $C_{shoot,dry}$ and $C_{root,dry}$ are shoot and root Cu or Cd concentration on dw basis, and $C_{iron,plaque}$ is the corresponding concentration of Cu or Cd in iron plaque.

All data were subjected to two-way analysis of variance (ANOVA) performed using the Microsoft Windows-based Genstat package (6th ed., NAG Ltd., England).

2 Results

2.1 Plant biomass

The dry weight of shoot and root differ greatly among genotypic cultivars ($p < 0.001$), with KY1360 being the highest and relatively lower for 94D-22, 94D-54, 94D-64 (Table 1). Soil Cu and Cd had very slight effect on root dw, but shoot dw showed some reduction in Cu treated soil.

2.2 Cu accumulation in plant tissues

Adding Cu into soil generally induced its accumulation in shoot and root, however, the extent of changes depends on the cultivars ($p < 0.001$). Compared to control, 100 mg/kg Cu treatment raised Cu concentrations by 3.0–4.6 fold in root and 1.2–2.9 fold in shoot (Table 2). KY1360 had the highest increment in root Cu while such change in shoot was observed in KY1360 and YY-1C. Although adding Cd into soil somehow reduced Cu content in shoot and root, but to a lesser degree than statistically significant.

jesc.ac.cn

Table 1 Biomass of different rice genotypes grown at Cu and Cd contaminated soils (g/pot)

Genotype	Shoot				Root			
	CK	Cu	Cd	Cu + Cd	CK	Cu	Cd	Cu + Cd
94D-22	3.15 ± 0.24	2.73 ± 0.19	2.56 ± 0.26	2.65 ± 0.51	0.86 ± 0.06	0.81 ± 0.07	0.70 ± 0.06	0.80 ± 0.15
94D-54	2.97 ± 0.14	2.15 ± 0.24	3.20 ± 0.35	2.52 ± 0.23	1.00 ± 0.07	0.86 ± 0.10	1.32 ± 0.16	0.88 ± 0.07
94D-64	2.92 ± 0.26	3.14 ± 0.23	2.83 ± 0.25	2.98 ± 0.33	1.00 ± 0.07	1.11 ± 0.12	1.00 ± 0.06	1.12 ± 0.13
Gui630	3.30 ± 0.36	3.28 ± 0.36	3.52 ± 0.44	3.12 ± 0.19	1.41 ± 0.11	1.46 ± 0.15	1.51 ± 0.14	1.42 ± 0.10
YY-1	3.33 ± 0.31	2.53 ± 0.15	2.59 ± 0.17	2.71 ± 0.35	1.14 ± 0.08	0.86 ± 0.02	0.93 ± 0.06	0.99 ± 0.08
KY1360	4.28 ± 0.41	3.64 ± 0.15	3.97 ± 0.47	3.95 ± 0.27	1.28 ± 0.11	1.15 ± 0.04	1.19 ± 0.14	1.32 ± 0.12
Analysis of variance								
Cu	$p < 0.05$				NS			
Cd	NS				NS			
Genotype	$p < 0.001$				$p < 0.001$			
Cu×Cd	NS				NS			
Cu×genotype	NS				NS			
Cd×genotype	NS				NS			

Data are expressed as mean ± standard errors; NS indicates the treatment effect not significant at the 0.05 level; CK is control, without Cu and Cd. These parameters express the same meaning in following tables.

Table 2 Concentrations of Cu in shoot and root of different rice genotypes grown in Cu and Cd contaminated soils (mg/kg)

Genotype	Shoot				Root			
	CK	Cu	Cd	Cu + Cd	CK	Cu	Cd	Cu + Cd
94D-22	14.44 ± 2.81	31.32 ± 4.87	11.37 ± 0.82	31.48 ± 5.69	21.21 ± 3.55	112.28 ± 13.16	24.33 ± 1.80	97.39 ± 9.60
94D-54	9.46 ± 0.29	28.34 ± 1.05	10.14 ± 0.76	18.95 ± 2.16	19.12 ± 0.80	76.03 ± 4.15	15.67 ± 0.49	84.47 ± 2.58
94D-64	9.63 ± 0.53	24.29 ± 1.73	9.36 ± 0.49	18.73 ± 1.02	19.31 ± 1.94	76.59 ± 6.35	17.61 ± 0.80	73.25 ± 1.26
Gui630	11.81 ± 0.24	34.40 ± 4.51	8.98 ± 0.74	33.24 ± 3.31	13.55 ± 0.55	70.11 ± 5.30	11.26 ± 0.47	66.06 ± 5.18
YY-1	7.91 ± 0.52	30.92 ± 2.62	7.74 ± 0.48	26.54 ± 2.04	15.69 ± 1.86	71.52 ± 5.65	13.86 ± 1.35	71.25 ± 6.90
KY1360	7.14 ± 0.23	27.80 ± 2.04	9.97 ± 0.88	20.58 ± 2.22	15.37 ± 2.18	86.76 ± 1.95	19.27 ± 2.29	71.31 ± 7.60
Analysis of variance								
Cu	$p < 0.001$				$p < 0.001$			
Cd	NS				NS			
Genotype	$p < 0.001$				$p < 0.001$			
Cu×Cd	NS				NS			
Cu×genotype	NS				$p < 0.01$			
Cd×genotype	NS				NS			

This result indicates that Cd has negligible influence on Cu accumulation.

2.3 Cd accumulation in plant tissues

Similarly, Cd concentration in shoot and root was higher in Cd treated soils ($p < 0.001$), however, inter-cultivar difference was not as significant as for Cu (Table 3). Compared to control, shoot and root Cd concentrations increased by 4.3–10.2 fold in shoot and 6.7–12.9 fold in root with 1.0 mg/kg Cd. KY1360 has the highest Cd content. Additional application of Cu into Cd treated soil enhanced Cd accumulation obviously ($p < 0.001$), suggesting an interactive relationship between these. However, the extent of interaction is dependent on the cultivars. Shoot Cd concentrations for Gui630, YY-1, 94D-22 and KY1360 in Cu + Cd combined treatment was respectively 110.6%, 77.0%, 58.6%, and 45.2% higher than that in Cd single application. Root Cd concentration was increased more pronounced in Gui630 (112.7%), YY-1 (51.2%), KY1360 (18.4%), and 94D-54 (17.9%). These results indicate that soil Cu can enhance Cd uptake and accumulation in rice.

2.4 Accumulation of P, Fe, Cu, and Cd in iron plaques of the six rice genotypes

Table 4 shows Cu and Cd concentrations in rice root surface iron plaques after separate and combined treatment. The accumulation of heavy metals in iron plaques is mainly determined by the genotypes. In addition, soil Cu treatment (100 mg/kg) led to 9.9–15.2 fold higher Cu concentrations in iron plaques with the highest value in YY-1 and 94D-22. Although adding Cd in soil had no impact on Cu status, the Cd 1.0 mg/kg was able to induce its own accumulation in iron plaques ($p < 0.001$). Compared to control, Cd treatment induced 0.5–2.6 fold higher concentration of the element depending on cultivars. In the combined contamination, Cu supplement had very minor influence on Cd concentration in iron plaques. For P and Fe, concentration of both elements is also varied greatly with cultivars. The Cd treatment had very little effect on P and Fe accumulation, and the Cu treatment showed very pronounced effect ($p < 0.01$) (Table 5).

2.5 Transference of Cu and Cd from iron plaques to plant tissues

F_{shoot} and F_{root} of Cu depend on cultivars and are affected by soil Cu treatment ($p < 0.001$), but not Cd. Under untreated condition, F_{shoot} and F_{root} values ranged between 3.2–5.9 and 6.8–10.8 in shoot and root respectively, both value reduced to 0.8–1.5 and 2.6–3.3 in Cu treated soil, and 0.9–1.2 and 2.4–4.3 in Cu + Cd combined treatment (Fig. 1).

F_{shoot} and F_{root} of Cd are also varied with cultivars, and affected by soil application of both Cd and Cu ($p < 0.001$). In the untreated soil, the F_{shoot} and F_{root} of Cd were 2.3–5.6

jesc.ac.cn

Table 3 Concentrations of Cd in shoot and root of different rice genotypes grown in Cu and Cd contaminated soils (mg/kg)

Genotype	Shoot				Root			
	CK	Cu	Cd	Cu + Cd	CK	Cu	Cd	Cu + Cd
94D-22	0.19 ± 0.02	0.30 ± 0.03	1.28 ± 0.09	2.03 ± 0.56	0.37 ± 0.07	0.63 ± 0.10	3.17 ± 0.41	3.56 ± 0.68
94D-54	0.24 ± 0.03	0.28 ± 0.04	2.01 ± 0.19	2.41 ± 0.03	0.57 ± 0.04	0.61 ± 0.08	4.52 ± 0.39	5.33 ± 0.36
94D-64	0.35 ± 0.01	0.38 ± 0.03	1.87 ± 0.24	2.30 ± 0.25	0.55 ± 0.07	0.66 ± 0.12	4.42 ± 0.61	4.40 ± 0.29
Gui630	0.21 ± 0.01	0.33 ± 0.06	1.32 ± 0.08	2.78 ± 0.53	0.41 ± 0.02	0.85 ± 0.15	3.14 ± 0.12	6.68 ± 1.15
YY-1	0.18 ± 0.01	0.22 ± 0.04	1.26 ± 0.10	2.23 ± 0.35	0.43 ± 0.03	0.49 ± 0.09	3.44 ± 0.29	5.20 ± 1.11
KY1360	0.13 ± 0.01	0.32 ± 0.02	1.46 ± 0.24	2.12 ± 0.25	0.27 ± 0.03	0.67 ± 0.04	3.76 ± 0.98	4.45 ± 0.17
Analysis of variance								
Cu	$p < 0.001$				$p < 0.001$			
Cd	$p < 0.001$				$p < 0.001$			
Genotype	NS				NS			
Cu×Cd	$p < 0.001$				$p < 0.05$			
Cu×genotype	NS				NS			
Cd×genotype	NS				NS			

Table 4 Concentrations of Cu and Cd in iron plaques of different rice genotypes grown in Cu and Cd contaminated soils

Genotype	Concentration of Cu (mg/kg)				Concentration of Cd (mg/kg)			
	CK	Cu	Cd	Cu + Cd	CK	Cu	Cd	Cu + Cd
94D-22	2.68 ± 0.28	41.07 ± 7.33	2.42 ± 0.13	27.21 ± 3.91	0.05 ± 0.01	0.11 ± 0.02	0.15 ± 0.02	0.16 ± 0.01
94D-54	2.04 ± 0.16	26.81 ± 1.14	1.99 ± 0.10	27.89 ± 1.82	0.05 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.22 ± 0.02
94D-64	2.72 ± 0.22	29.57 ± 2.83	2.61 ± 0.30	17.13 ± 1.17	0.06 ± 0.00	0.03 ± 0.00	0.19 ± 0.02	0.10 ± 0.01
Gui630	2.04 ± 0.21	22.45 ± 0.69	1.80 ± 0.23	27.86 ± 2.70	0.04 ± 0.01	0.04 ± 0.01	0.11 ± 0.01	0.10 ± 0.01
YY-1	1.44 ± 0.12	23.38 ± 2.55	2.12 ± 0.12	24.16 ± 2.85	0.06 ± 0.01	0.06 ± 0.00	0.18 ± 0.03	0.13 ± 0.03
KY1360	2.28 ± 0.19	26.58 ± 1.34	2.83 ± 0.38	23.65 ± 3.70	0.06 ± 0.01	0.04 ± 0.00	0.16 ± 0.02	0.07 ± 0.01
Analysis of variance								
Cu	$p < 0.001$				NS			
Cd	NS				$p < 0.001$			
Genotype	$p < 0.01$				$p < 0.001$			
Cu×Cd	$p < 0.05$				NS			
Cu×genotype	$p < 0.05$				$p < 0.001$			
Cd×genotype	$p < 0.05$				NS			

Table 5 Concentrations of P and Fe in iron plaques of different rice genotypes grown in Cu and Cd contaminated soils

Genotype	Concentration of P (g/kg)				Concentration of Fe (g/kg)			
	CK	Cu	Cd	Cu + Cd	CK	Cu	Cd	Cu + Cd
94D-22	0.44 ± 0.10	0.60 ± 0.05	0.49 ± 0.00	0.47 ± 0.14	13.89 ± 3.58	18.44 ± 2.20	14.33 ± 1.37	14.96 ± 4.50
94D-54	0.34 ± 0.04	0.53 ± 0.05	0.39 ± 0.03	0.46 ± 0.46	10.58 ± 2.09	18.24 ± 2.46	9.75 ± 0.74	11.51 ± 0.71
94D-64	0.48 ± 0.03	0.46 ± 0.03	0.52 ± 0.05	0.35 ± 0.00	7.70 ± 1.23	10.71 ± 1.21	12.48 ± 2.81	11.21 ± 0.72
Gui630	0.33 ± 0.04	0.32 ± 0.08	0.41 ± 0.04	0.38 ± 0.05	5.62 ± 0.88	10.84 ± 3.26	7.14 ± 0.95	7.74 ± 2.53
YY-1	0.28 ± 0.01	0.60 ± 0.03	0.65 ± 0.07	0.55 ± 0.09	9.12 ± 0.98	19.53 ± 2.13	13.66 ± 1.21	13.84 ± 2.79
KY1360	0.48 ± 0.07	0.35 ± 0.04	0.41 ± 0.08	0.39 ± 0.04	10.41 ± 1.12	9.89 ± 1.63	8.84 ± 2.46	8.60 ± 1.08
Analysis of variance								
Cu	NS				$p < 0.01$			
Cd	NS				NS			
Genotype	$p <0.01$				$p < 0.001$			
Cu×Cd	$p < 0.01$				$p < 0.01$			
Cu×genotype	$p < 0.05$				NS			
Cd×genotype	NS				NS			

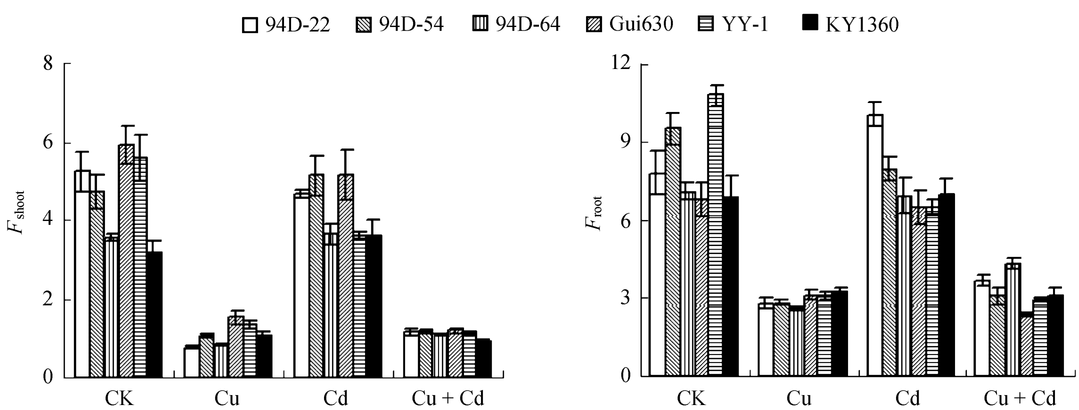

Fig. 1 Transfer factor of Cu from rice root surface to root and shoot. Bar expresses standard error.

jesc.ac.cn

and 4.8–11.0, respectively. However, F_{shoot} was as high as 2.9–11.6, 7.9–12.8 and 11.5–29.4, and F_{root} was 6.2–25.3, 20.5–30.9 and 22.3–62.1 with the treatments of Cu, Cd, and Cu + Cd, respectively (Fig. 2).

Under combined contamination, the F_{root} and F_{shoot} of Cd in the range of 22.3–62.1 and 11.5–29.4, however, F_{root} and F_{shoot} of Cu were 2.4–4.3 and 0.9–1.2, respectively. These results indicated that Cd moves more readily than Cu from iron plaques to root and shoot. The other parameters also showed similar trend.

3 Discussion

In rice plants, Cu concentration was largely dependent on the genotypic cultivars ($p < 0.001$), however, such effect was not observed for Cd. In shoot tissue, the maximum inter-cultivar difference in Cu concentration was as high as 102.2%, 41.6%, 46.9% and 77.5% in shoot, and 56.5%, 60.1%, 116.1% and 47.4% in root (Table 2) under the treatments of CK, Cu, Cd and Cu + Cd, respectively. Similar results have been obtained from other studies. In South Australia, McLaughlin *et al*. (1994) compared and observed big difference in Cd uptake and accumulation in 14 potato genotypes, for some cultivar tubers Cd content was in the range of 30–50 μg/kg fresh weight while others are much higher than the national regulation level (50 μg/kg fresh weight). Based on this observation, it was subsequently proposed to screen for low Cd cultivars to avoid its harmful effect to human health. In another study some high and low accumulation lettuce cultivars were selected after comparing their uptake and accumulation of Cd, Cu, Pb and Zn (Crews and Davies, 1985).

In the soil-plant system, the interaction between heavy metals can affect their individual function. However, this interaction relationship can be very complex in different soil and plant environments. In this study, soil application of 1.0 mg/kg Cd had a negligible effect on Cu uptake by rice plants, however, Cu treatment at 100 mg/kg Cu greatly enhanced Cd accumulation ($p < 0.001$). This can be caused by that under the experimental condition, Cu and Cd absorb to the soil colloids competitively which result in more Cd in soil solution available to the plants. Similarly, Fargasova (2001) also found that Zn, Cu and Fe all have some additive effect for Cd accumulation. On the other hand, Cd was observed to enhance Zn and Cu uptake by mustard cotyledons.

However, different heavy metals can become antagonistic under certain conditions. Fritioff and Greger (2006) studied the accumulation of heavy metal (Zn, Cu, Cd and Pb) by aquatic plant *Potamogeton natans*, and found that all other elements can reduce the Cd concentration in root, but leaf Cu concentration was increased. The giant fresh water plant *Eriocaulon septangulare*, also exhibited the inhibitory effect of Cd uptake by other heavy metals when grown in heavy metal contaminated water zone (Stewart and Malley, 1999). Comparing Cd, Pb, and Cu accumulation by three oil crop species (dill, peppermint, and basil) found that peppermint and basil have much lower Cd level when treated with combined contamination of Cd-Pb, Cd-Cu, and Cd-Pb-Cu compared to Cd single treatment. This inhibitory effect on Cd uptake is caused by the competitive binding between Pb and Cu with Cd in the growth medium (Zheljazkov *et al*., 2006). Similar mode of antagonistic interaction among Cd, Cu and Pb has been observed earlier in different plant species (Kabata-Pendias and Pendias, 1991).

Many studies have shown that Cd can be easily transferred from root to shoot (Alloway, 1990; Kabata-Pendias and Pendias, 1991). We also noticed that Cd moves more readily from the iron plaques into root and shoot. Raising Cd level can result in the reduction in the transfer rate of Cd from root to shoot (Cunningham *et al*., 1975; Zheljazkov *et al*., 2006). In contrast, Cd treatment induced high F_{shoot} and F_{root} of Cd, and the opposite for the Cu treatment. The results indicate that high Cd level enhances Cd movement from iron plaques to root and shoot, but Cu did not have similar effect.

Formation of iron plaque is a natural phenomenon on

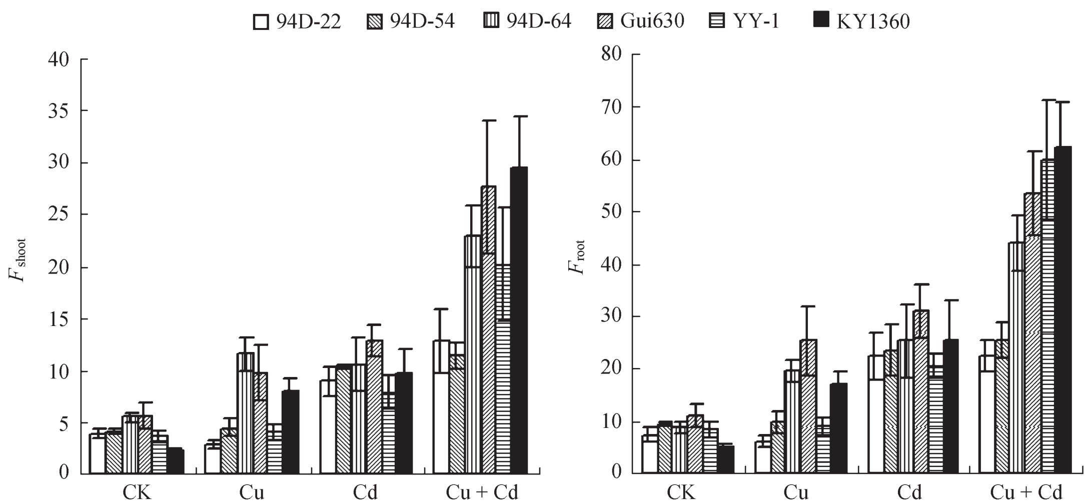

Fig. 2 Transfer factor of Cd from rice root surface to root and shoot. Bar expresses standard error.

jesc.ac.cn

rice root surface. Such structure can also be found in other species such as submerged plants, emerged plants, continental plants in water logging soil, non-flowering plants, conifers. The function of the iron plaques upon heavy metal absorption depends on the elements. In this study, we have found that rice root surface iron plaques can absorb a large amount of Cu and Cd. The big species-specific difference observed in this study might be related to the thickness of the iron plaques. Furthermore, this study also revealed that iron plaques can not reduce the transfer of Cu and Cd into plants, moreover, Cd can be more easily absorbed into root and shoot compared to Cu (Figs. 1 and 2). Rice with iron plaques can tolerant Cu and Ni toxicity stronger than those without such structure. However, iron plaques do not interfere with root uptake of Cu. The reason for the oxide membrane to reduce Cu toxicity is probably because of the Cu passivation by Fe in root cortex.

The iron plaques can absorb and precipitate some heavy metals and thus exclude the elements outside of the plants. In addition, these iron plaques can provide a large amount of Fe to plants competing for the sensitive binding sites with heavy metals. *Phragmites communis* Trin. forms thicker iron plaques when grown at pH 6.0, and these plaques can serve as barriers for Cu and Mn. At pH 3.5, the plaques become thinner and therefore lose the function to block the Cu and Mn absorption (Batty *et al*., 2000). It is also reported that the rice root surface iron plaques can strongly enrich the arsenate by acting as the buffer zone of the toxic ions in the root microenvironment and thereby prevent it from moving into rice root (Liu *et al*., 2004; Chen *et al*., 2005).

The fact that iron plaques to absorb metal at varied efficiency possibly related with their physiological and chemical properties. X-ray fluorescent microcopy sectioning profile analysis found that root surface iron plaques mainly consist of oxides of iron and manganese as well as their hydroxides (Batty *et al*., 2000). These structures have special electro-chemical properties and belong to amphoteric colloid. Activities on the colloidal membrane such as ion exchange, oxidoreduction, organic and inorganic complexation reaction can all change the status of the heavy metals in root zone, and thereby affect their biological toxicity (Otte *et al*., 1989; St-Cyr and Crowder, 1990).

4 Conclusions

The biomass yield is mainly dependent on the genotypes, with the highest from KY1360 and the lowest from 94D-22 and 94D-54. Root growth remained similar upon the treatment of Cu and Cd. Cu reduced shoot dry weight.

Both single and combined applications promoted the accumulation of Cu and Cd in shoot and root. Cu induced Cd accumulation, however, the opposite was not true. Soil supplement with Cu/Cd increased their concentrations in rice root surface iron plaques. Cu significantly affected Fe but not Cd concentrations in iron plaques, however, similar result was not observed for Cu, P and Fe upon exposure to Cd treatment.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 30671204, 40620120436), the Tianjin Specific Fund for Scientific and Technologic innovation (No. 06FZZDSH00900), and the Hi-Tech Research and Development program (863) of China (No. 2007AA061001).

References

Alexander P D, Alloway B J, Dourado A M, 2006. Genotypic variations in the accumulation of Cd, Cu, Pb and Zn exhibited by six commonly grown vegetables. *Environmental Pollution*, 144: 736–745.

Ali N A, Bernal M P, Ater M, 2004. Tolerance and bioaccumulation of cadmium by *Phragmites australis* grown in the presence of elevated concentrations of cadmium, copper, and zinc. *Aquatic Botany*, 80: 163–176.

Alloway B J, 1990. Heavy Metals in Soils. Glasgow: Blackie. 321.

Balsberg Påhlsson A M, 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. A literature review. *Water, Air and Soil Pollution*, 47: 287–319.

Batty L C, Baker A J M, Wheeler B D, Curtis C D, 2000. The effect of pH and plaque on the uptake of Cu and Mn in *Phragmites australis* (Cav.) Trin ex Steudel. *Annals of Botany*, 86: 647–653.

Boggess S F, Willavize S, Koeppe D E, 1978. Differential response of soybean genotypes to soil cadmium. *Agronomy Journal*, 70: 756–760.

Cao Z H, Hu Z Y, Wong M H, 2000. Copper contamination in paddy soils irrigated with wastewater. *Chemosphere*, 41: 3–6.

Carrier P, Baryla A, Havaux M, 2003. Cadmium distribution and microlocalization in oilseed rape (*Brassica napus*) after long-term growth on cadmium-contaminated soil. *Planta*, 216: 939–950.

Chatterjee J, Chatterjee C, 2000. Phytotoxicity of cobalt, chromium and copper in cauliflower. *Environmental Pollution*, 109: 69–74.

Chen H M, 1996. Heavy Metal Contamination in the Soil-Plant System. Beijing: Scientific Press. 71–85.

Chen Z, Zhu Y G, Liu W J, Meharg A A, 2005. Direct evidence showing the effect of root surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa* L.) roots. *New Phytologist*, 165: 91–97.

Costa G, Morel J L, 1994. Efficiency of H^+-ATPase activity on cadmium uptake by four cultivars lettuces. *Journal of Plant Nutrition*, 17: 627–637.

Crews H M, Davies B E, 1985. Heavy metal uptake from contaminated soils by six varieties of lettuce (*Lactuca sativa* L.). *Journal of Agricultural Science (Cambridge)*, 105: 591–595.

Cunningham L M, Collins F W, Hutchinson T C, 1975. Physiological and biochemical aspects of cadmium toxicity in soybean. In: Proceedings of the International Conference Heavy Metals in the Environment, Toronto, October 27. 97–103.

Das P, Samantaray S, Rout G R, 1997. Studies on cadmium toxicity in plants: A review. *Environmental Pollution*, 98: 29–36.

Fargasova A, 2001. Effect of Cd in combination with Cu, Zn, Pb and Fe on root prolongation and metal accumulation in the

jesc.ac.cn

roots and cotyledones of mustard (*Sinapis alba*) seedlings. Plant, *Soil and Environment*, 47: 97–103.

Florijn P J, van Beusichem M L, 1993. Uptake and distribution of cadmium in maize inbred lines. *Plant Soil*, 150: 25–32.

Franklin N M, Stauber J L, Lim R P, Petocz P, 2002. Toxicity of metal mixtures to a tropical freshwater alga (*Chlorella* sp.): The effect of interactions between copper, cadmium, and zinc on metal cell binding and uptake. *Environmental Toxicology and Chemistry*, 21: 2412–2422.

Fritioff Å, Greger M, 2006. Uptake and distribution of Zn, Cu, Cd, and Pb in an aquatic plant *Potamogeton natans*. *Chemosphere*, 63: 220–227.

Grant C A, Bailey L D, McLaughlin M J, Singh B R, 1999. Management factors which influence cadmium concentrations in crops. In: Cadmium in Soils and Plants (McLaughlin M J, Singh B R, eds.). Dordrecht: Kluwer Academic Publishers. 151–198.

Hazardous Waste Consultant, 1996. Remediating Soil and Sediment Contaminated with Heavy Metals. The Netherlands: Elsevier Science.

John M K, Laerhoven N, 1976. Differential effects of cadmium on lettuce varieties. *Environmental Pollution*, 10: 163–173.

Kabata-Pendias A, Pendias H, 1991. Trace Elements in Soils and Plants (2nd ed.). Boca Raton-Ann Arbor-London: CRC Press.

Liu W J, Zhu Y G, Hu Y, Williams P N, Gault A G, Meharg A A *et al*., 2006. Arsenic sequestration in iron plaque, its accumulation and speciation in mature rice plants (*Oryza sativa* L.). *Environmental Science and Technology*, 40: 5730–5736.

Liu W J, Zhu Y G, Smith F A, Smith S E, 2004. Do phosphorus nutrition and iron plaque alter arsenate (As) uptake by rice seedlings in hydroponic culture? *New Phytologist*, 162: 481–488.

Lu R S, 1999. Agricultural Chemical Analytical Methods of Soil. Beijing: Chinese Agricultural Scientific Press.

McLaughlin M J, Williams G M J, Mckay A, Kirkham R, Gunton J, Jackson K J *et al*., 1994. Effect of cultivar on uptake of cadmium by potato tubers. *Australian Journal of Agricultural Research*, 45: 1483–1495.

Meharg A A, Jardine L, 2003. Arsenite transport into paddy rice (*Oryza sativa*) roots. *New Phytologist*, 157: 39–44.

Murakami M, Ae N, Ishikawa S, 2007. Phytoextraction of cadmium by rice (*Oryza sativa* L.), soybean (*Glycine max* (L.) Merr.), and maize (*Zea mays* L.). *Environmental Pollution*, 145: 96–103.

Oliver D P, Hannam R, Tiller K G, Wilhelm N S, Merry R H, Cozens G D, 1994. The effects of zinc fertilization on cadmium concentration in wheat grain. *Journal of Environmental Quality*, 23: 705–711.

Otte M L, Rozema J, Koster L, Haarsma M S, Broekman R A, 1989. Iron plaque on roots of *Aster tripolium* L.: interaction with zinc uptake. *New Phytologist*, 111: 309–317.

Sarret G, Saumitou-Laprade P, Bert V, Proux O, Hazemann J L, Traverse A S, Marcus M A, Manceau A, 2002. Forms of zinc accumulated in the hyperaccumulator *Arabidopsis halleri*. *Plant Physiology*, 130: 1815–1826.

St-Cyr L, Crowder A A, 1990. Manganese and copper in the root plaque of *Phragmites australis* (Cav.) Trin.ex Steudel. *Soil Science*, 149: 191–198.

Stewart A R, Malley D F, 1999. Effect of metal mixture (Cu, Zn, Pb, and Ni) on cadmium partitioning in littoral sediments and its accumulation by the freshwater macrophyte *Eriocaulon septangulare*. *Environmental toxicology and chemistry*, 18: 436–447.

Tang C, Robson A D, 2000. Cultivar variation in the effect of chlorsulfuron in depressing the uptake of copper in wheat. *Plant and Soil*, 225: 11–20.

Wang H Y, 2003. Effect of Cd, Zn, and Pb compound pollution on celery in a ferric acrisol. *Soil and Sediment Contamination*, 12: 357–370.

Wu F B, Zhang G P, 2002. Genotypic difference in effect of cadmium on growth and mineral concentrations in barley seedlings. *Bulletin of Environmental Contamination and Toxicology*, 69: 219–227.

Zhang G P, Fukami M, Sekimoto H, 2000. Genotypic difference in effects of cadmium on growth and nutrition compositions in wheat. *Journal of Plant Nutrition*, 23: 1337–1350.

Zheljazkov V D, Craker L E, Xing B S, 2006. Effects of Cd, Pb, and Cu on growth and essential oil contents in dill, peppermint, and basil. *Environmental and Experimental Botany*, 58: 9–16.

jesc.ac.cn