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Cadmium Pollution in Paddy Soil as Affected by Different Rice (*Oryza sativa* L.) Cultivars

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Cadmium is principally dispersed in natural and agricultural environments through various agricultural, mining and industrial activities as well as resulting from the exhaust gases of automobiles (Das et al., 1997). This trace metal is pollutant and potential toxin that has no known function in any biological organism, and is one of the most dangerous heavy metals for the environment due to its high mobility and low toxic concentration in organisms. Cadmium in soils is readily taken up by plants and is not phytotoxic at concentration in crops that can significantly increase human exposure (Adriano, 1986). Excessive exposure to Cd has been associated with various illnesses in humans, including gastroenteritis, renal tubular dysfunction, hypertension, cardiovascular disease, pulmonary emphysema, cancer, and osteoporosis (Wagner, 1993). For example, elevated levels of Cd in the diet and drinking water were concluded to be causative factors in the 1964 occurrence of Itai Itai disease (severe osteoporosis/osteomalacia and renal tubular dysfunction) in the Toyoma Prefecture in Japan (Hallenbeck, 1984). Cadmium absorption, accumulation and distribution in food crops such as rice, wheat, barley, maize and potato (Devkota and Schmidt, 2000; Grant and Bailey, 1996; Jeng and Singh, 1995; Maier et al., 1996; McLaughlin et al., 1994; Moraghan, 1993; Oliver et al., 1995; Ramachandran and Souza, 1998; Yang, 1999; Zhou et al., 1994), vegetable crops such as Chinese cabbage, winter greens, celery and cucumber (Ni et al., 2002; Moreno-Caselles et al., 2000), oil seed crops such as sunflower and flaxseed crops (Grant and Bailey, 1997; Simon, 1998), and forage crops (Singh and Nayyar, 1994) had been generally studied. However, genotypic differences of cadmium accumulation in food crops, especially in rice plants is still not informed.

The objective of the reported work was to study the impact of cadmium addition on the growth of selected rice genotypes, and to identify genotypic differences of cadmium accumulation and distribution in rice plants. And the criteria of Cd pollution in soil based on the hygienic limit of Cd in rice was evaluated.

MATERIALS AND METHODS

Before the pot experiment, the topsoil (0~20 cm) of a flooded rice soil, collected

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from Jiaxing city, Zhejiang province, P. R. China, as the initial soil was incubated with different doses of Cd (2.0, 4.0, 6.0, 8.0, 10.0 mg Cd/kg soil) added as $Cd(NO_3)_2 \cdot 4H_2O$ under the flooding condition for 12 weeks. The transformation of added Cd in the same soil became equilibrated after 6-weeks incubation (Diao, 2004, data unpublished). Martinez and Motto (2000) reported that the transformation of heavy metals added as single solutions in mineral soils could get balance after 40-days incubation. After 12 weeks incubation, the soils were air-dried. Thus, the initial soil and incubated soils with different total Cd contents (0.432 mg/kg soil in the initial soil, 2.432, 4.432, 6.432, 8.432, 10.432 mg/kg soil in the incubated soils) were prepared for the pot experiment.

Seeds of five rice cultivars as Huapei 528 (C1), Zhenongda 454 (C2), Xinzaozhan (C3), Xijing 7 (C4) and Zhefu 910 (C5) were germinated on wetted filter paper for two days in the dark (25°C). The germinated seeds were sowed on quartz sand with Cd-free nutrient solution for preparing seedlings. The nutrient solution contained the following nutrient concentrations (mg/L): N 40 (in NH_4NO_3), P 10 (in $NaH_2PO_4 \cdot 2H_2O$), K 40 (in K_2SO_4), Ca 40 (in $CaCl_2$), Mg 40 (in $MgSO_4 \cdot 7H_2O$), Mn 0.5 (in $MnCl_2 \cdot 4H_2O$), Mo 0.05 [in $(NH_4)_6Mo_7O_{24}$], B 0.2 (in H_3BO_3), Zn 0.01 (in $ZnSO_4 \cdot 7H_2O$), Cu 0.01 (in $CuSO_4 \cdot 5H_2O$), Fe 2.0 (in $FeCl_3 \cdot 2H_2O$ + citric acid).

A pot experiment was performed on the prepared soils grown with selected rice cultivars of Huapei 528, Zhenongda 454, Xinzaozhan, Xijing 7 and Zhefu 910, respectively. The initial soil used in this experiment had the following characteristics: $pH_{(H2O)}$ 6.89, organic C 30.1 g/kg soil, total N 2.43 g/kg soil, total P 0.75 g/kg soil, total K 16.8 g/kg soil, total Cd 0.432 mg/kg soil, hydrolizable N 128.4 mg/kg soil, available P 14.2 mg/kg soil, exchangeable K 126.4 mg/kg soil and extractable Cd 0.031 mg/kg soil.

The initial soil and five incubated soils with different total Cd were used as the treatments. Six thousand gram of the prepared soils was placed in each plastic pot. The doses of N (in urea), P (in potassium dihydrophosphate) and K (in potassium dihydrophosphate and potassium chlorite) supplied were equal for all treatments in the pot experiment: 0.2 N, 0.1 P_2O_5 and 0.2 K_2O g/kg soil. The treatments were trireplicated and randomly arranged. Sixteen seedlings of similar length were selected and transplanted into each plastic pot. During the experimental period, deionized water was added to compensate for evapotranspiration when it was needed. Plants were harvested at mature stage and washed with deionized water.

The important characteristics of the soils were measured with conventional methods (Committee of Agrochem, Soil Sci Soc of China, 1983). Plant tissues (straw and brown rice) were oven-dried at 60°C to constant weight and ground in a stainless steel mill. Dry plant material was placed in porcelain crucibles and mineralized by oven-drying digestion methods. Digested samples were dissolved, filtered and brought to 25-mL with 0.5ml/L HNO_3. The extractable Cd in soils was extracted by 0.005mol/L DTPA. Cadmium in extracting solution of soils and digesting solution of plant materials was measured by atomic absorption spectrophotometer (Committee of Agrochem, Soil Sci Soc of China, 1983).

Table 1. Biomass production of rice genotypes (ns = non-significant).

Soil Cd content (mg/kg)	Straw biomass (g/pot)				
	C1	C2	C3	C4	C5
0.432	24.11	18.59	20.01	25.49	17.76
2.432	20.63	14.95	21.3	25.16	17.66
4.432	23.26	16.18	23.51	25.46	15.12
6.432	22.60	18.02	24.81	28.28	17.52
8.432	27.10	17.86	20.00	28.44	15.31
10.432	25.21	18.63	25.11	27.35	17.12
F-anova	ns	ns	ns	ns	ns
	Grain biomass (g/pot)				
	C1	C2	C3	C4	C5
0.432	5.25	8.80	3.26	2.38	2.32
2.432	3.05	6.59	3.28	4.73	2.77
4.432	4.01	7.47	3.15	5.53	1.95
6.432	6.47	9.63	2.48	3.86	5.12
8.432	2.73	8.09	2.64	6.11	3.36
10.432	5.33	9.27	4.05	5.32	2.74
F-anova	ns	ns	ns	ns	ns

The significance of differences between the means of the treatments was evaluated by one-way analysis of variance followed by Duncan multiple range test at 5% significance. Linear regression analysis was performed to establish linear relations between Cd concentrations in plant tissues and total Cd contents in soils, as well as between Cd concentrations in straws and brown rice.

RESULTS AND DISCUSSION

No nutrient deficiency nor toxicity symptoms were visible on the tested rice cultivars in the pot experiment. Biomass production of straws and grains, expressed as dry weight, were not significantly different among the treatments (Table 1). This result indicated that Cd concentrations of the treatments established in this study were likely to be below phytotoxic levels for the tested rice cultivars. Sarkunan et al. (1987) reported that significant reduction in rice yields occurred with 150 mg Cd /kg soil. Therefore, rice plants with the general tolerance to cadmium grown in Cd-polluted soil have potential to contribute substantial Cd to the human diet.

Cadmium concentrations in straw and brown rice of five rice cultivars from the

Table 2. Cadmium concentration in brown rice and straw of test rice genotypes (expressed in dry weight basis).

Soil Cd content (mg/kg)	Cd concentration in straws (mg/kg DW)				
	C1	C2	C3	C4	C5
0.432	0.55	2.87	2.98	0.62	0.73
2.432	3.09	4.47	5.43	2.61	3.35
4.432	4.20	9.04	11.88	5.32	7.47
6.432	10.44	11.09	14.80	6.63	10.48
8.432	12.61	13.77	19.84	9.48	12.14
10.432	15.13	15.11	22.03	10.37	13.39
F-anova	**	**	**	**	**
	Cd concentration in brown rice (mg/kg DW)				
	C1	C2	C3	C4	C5
0.432	0.08	0.17	0.45	0.30	0.22
2.432	0.42	0.41	0.76	0.92	0.41
4.432	0.72	0.91	1.93	1.39	0.86
6.432	1.91	1.22	2.65	2.26	2.71
8.432	2.63	1.79	4.74	2.67	3.05
10.432	3.63	2.36	5.04	3.59	3.54
F-anova	**	**	**	**	**

** Represents significant differences between values at p=0.01, by Anova Test.

Table 3. Correlative relationships between Cd concentration in straw (C_{st}) and in brown rice (C_{br}).

Cultivars	Correlative equation	Correlative coefficient	Significance
Huapei 528	$C_{br} = 0.2143\ C_{st}$	0.982 (n=6)	**
Zhenongda 454	$C_{br} = 0.1293\ C_{st}$	0.948 (n=6)	**
Xinzaozhan	$C_{br} = 0.2133\ C_{st}$	0.968 (n=6)	**
Xijing 7	$C_{br} = 0.3161\ C_{st}$	0.977 (n=6)	**
Zhefu 910	$C_{br} = 0.2392\ C_{st}$	0.943 (n=6)	**

** Represents that the correlative relationship is significant at the level of 1%.

pot experiment were presented in Table 2. Cadmium concentrations on the dry weight basis were 0.55-15.13, 2.87-15.11, 2.98-22.03, 0.62-10.37, 0.73-13.39 mg/kg dry weight for the straws, and 0.08-3.63, 0.17-2.36, 0.45-5.04, 0.30-3.59, 0.22-3.54 mg/kg dry weight for the brown rice of Huapei 528, Zhenongda 454,

Table 4. Correlative relationships between Cd concentration in straw (C_{st}) or in brown rice (C_{br}) and total Cd content in soil (C_s) and the evaluated criteria of Cd pollution in soil.

Cultivars	Correlative relationships C_{st} vs C_s			
	Correlative equation	Correlative coefficient	Significance	
Huapei 528	$C_{st} = 1.448\ C_s$	0.981 (n=6)	**	
Zhenongda 454	$C_{st} = 1.608\ C_s$	0.949 (n=6)	**	
Xinzaozhan	$C_{st} = 2.265\ C_s$	0.982 (n=6)	**	
Xijing 7	$C_{st} = 1.056\ C_s$	0.992 (n=6)	**	
Zhefu 910	$C_{st} = 1.421\ C_s$	0.979 (n=6)	**	
	Correlative relationships C_{br} vs C_s			The criteria of Cd concentration (mg/kg soil)
	Correlative equation	Correlative coefficient	Significance	
Huapei 528	$C_{br} = 0.3100\ C_s$	0.963 (n=6)	**	0.645
Zhenongda 454	$C_{br} = 0.2127\ C_s$	0.991 (n=6)	**	0.940
Xinzaozhan	$C_{br} = 0.4862\ C_s$	0.975 (n=6)	**	0.411
Xijing 7	$C_{br} = 0.3361\ C_s$	0.994 (n=6)	**	0.595
Zhefu 910	$C_{br} = 0.3435\ C_s$	0.957 (n=6)	**	0.582

** Represents that the correlative relationship is significant at the level of 1%.

Xinzaozhan, Xijing 7 and Zhefu 910 in order. The order of the five test rice cultivars for cadmium accumulation in brown rice was Xinzaozhan > Huapei 528 ≈ Xijing 7 ≈ Zhefu 910 > Zhenongda 454. The differences on cadmium accumulation among culture varieties or inbred lines of the same crop (Florijn et al., 1992; Florijn and Van Beusichen, 1993; Garate et al., 1993) were commonly reported. For each rice cultivar, Cd concentration in brown rice was obviously lower than that in straw at the same Cd level. This evolution was a common observation in rice plants (Hsieh, 1990; Liu et al., 2003). It was also found that Cd concentration in brown rice was positively correlative to Cd concentration in straw with the regression coefficients as 0.982** for Huapei 528, 0.948** for Zhenongda 454, 0.968** for Xinzaozhan, 0.977** for Xijing 7 and 0.943** for Zhefu 910 (Table 3). According to the correlative equation, the order of C_{br}/C_{st} ratio (ratio of Cd concentration in brown rice to Cd concentration in straw) was as Xijing 7 > Huapei 528 ≈ Xinzaozhan ≈ Zhefu 910 > Zhenongda 454. This results implied that Xijing 7 had the highest potential of Cd translocation from straw to brown rice, and Zhenongda 454 had the lowerest potential of Cd translocation from straw to brown rice.

From Table 2, it could be also observed that cadmium concentration in straw and brown rice increased with the increasing of cadmium concentration in soil. The results of statistical analysis indicated that cadmium concentration in straw or brown rice was positively correlative to total Cd content in soil. The linear equations illustrating the correlative relationships and their regression coefficients were presented in Table 4. In many cases, a linear relationship between Cd in plant material versus Cd in growth medium was reported (Filip et al., 1998; Kabata-Pendias and Pendias, 1984, Ni et al., 2002). As the regression coefficients were significant at the level of 1%, the regression equation of Cd concentration in brown rice versus Cd concnetration in soil could be used to estimate the criteria of cadmium concentration in soil with the limit of cadmium concentration in rice (0.2 mg/kg dry weight, GBN 238-84 in China). The calculated results (showed in Table 4) indicated that the criteria for total Cd content in soil are 0.645, 0.940, 0.411, 0.595, 0.582 mg/kg soil for Huapei 528, Zhenongda 454, Xinzaozhan, Xijing 7 and Zhefu 910 in order, understanding that the threshold for total Cd content in soil is 0.4mg/kg soil, which is lower than the limit of total soil Cd content (0.6mg/kg soil, GB 15618-1995 in China). On the other hand, Zhenongda 454 could be regarded as lower Cd-accumulating genotype, as it is with lower potential of Cd translocation from straw to brown rice and lower capacity for Cd uptake from soil, and allowed the threshold for total Cd content in soil up to 0.9mg/kg soil.

Cadmium did not affect the growth of rice at the level up to 10.432 mg/kg soil as total Cd of soil in the pot experiment. Cadmium concentrations in straw and brown rice of tested rice genotypes were linearly responded to the Cd levels in soil. Based on the regression equations established in this study and the limit of cadmium concentration in rice, the threshold of Cd concentration for soil was evaluated as 0.4 mg/kg soil of total Cd. The lower Cd-accumulating rice genotypes such as Zhenongda 454 identified in this study may catch the need for utilization of slightly Cd-polluted soil. And the high capacity for cadmium accumulation in brown rice of most rice genotypes together with the absence of visual symptoms points to a potential danger for humans.

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