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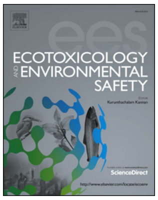

Effect of combined application of lead, cadmium, chromium and copper on grain, leaf and stem heavy metal contents at different growth stages in rice

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ABSTRACT

Most studies on plants' response to heavy metal toxicity have been focusing on single metals. However, soils are always contaminated by several kinds of heavy metals. In this study, pot experiments were carried out to investigate the effects of combined toxicity on two rice genotypes differing in Cd accumulation (Xiushui817, a low-grain-Cd-accumulation and Zheda821, a high-grain-Cd-accumulation genotype). Yield, heavy metal concentrations of grain and leaf/stem at different growth stages were measured under combined application of Cd, Cr, Pb and Cu. Yield was significantly decreased under higher Pb and Cd treatment in both genotypes with Xiushui817 showing greater reduction. Increasing soil Cu level showed no significant effect on grain yield. Zheda821 consistently showed a higher grain Cd content than Xiushui817. The application of Pb, Cd, Cr and Cu significantly affected grain Cd, Cr and Cu accumulations. Similar trends were also observed in leaves and stems at harvest stage. The critical levels of leaf/stem Cd and Cr for safe rice production were also estimated. Alleviation measures should be taken to decrease Cd or Cr accumulations in grain of rice if leaf or stem Cd or Cr concentrations at different growth stages exceed the critical levels.

1. Introduction

Soil heavy metal (HM) pollution has become a world-wide environmental issue and is growing as a result of rapid industrialization and other anthropogenic activities (Chen et al., 2010; Anjum et al., 2015, 2016). Excessive intake of HMs by humans can cause serious health problems, such as bone damage, kidney disease, renal tubular dysfunction and even death (Loganathan et al., 2012; Byber et al., 2016). About 10 million ha of arable land in China have been contaminated, and about 12 million tonnes of grains are polluted by HMs in soil each year (Chen et al., 2015). In China, Pb, Cd and Cr are the key heavy metal pollutants and more than 1.5 million sites are exposed by heavy metal (Hu et al., 2014). The discharged heavy metals in solid wastes, waste water and gas are about 900,000 t each year from 2005 to 2011 (Hu et al., 2014). Zhuang et al. (2009) found that the content of copper (Cu), lead (Pb) and cadmium (Cd) in paddy soil exceeded the maximum permissible concentrations for Chinese agricultural soils around the Dabaoshan mine, south China. Excessive HM concentrations in soils results in various problems in crops such as disturbances of photosynthesis, respiration, energy transduction, protein synthesis, redox equilibrium and ion homeostasis resulting in poor quality products and reduced yields. In addition, the HMs enter the food chain and may affect human and animal health (Jaishankar et al., 2014). Moreover, recently, it has been widely recognized that HMs exhibit their toxicity not only as individual entities but also as mixtures (Wang and Fowler, 2008); the co-exposure of HMs exerts even more toxicity on crops (Guo et al., 2007).

Rice, as one of the most important food cereals, is one of the major sources of HMs intakes for humans (Kikuchi et al., 2008). Therefore, for safe food production, there is an urgent need to decrease HM accumulation in grains of rice. Until recently, little research has been conducted on the combined effects of HMs on plant growth and the consequences for human health. So, this study is needed and conducted, and used two different Cd-accumulation rice genotypes (Cao et al., 2014). Significant differences in grain HM accumulation can be found among different genotypes of crops. HMs are absorbed from soil and accumulate in the edible parts of the crops after several sophisticated processes of transport. There are four major transport processes for heavy metal accumulation in rice: root uptake; root-to-shoot translocation; redirection at nodes; remobilization from leaves (Uraguchi and Fujiwara, 2012). The differential accumulation level of heavy metal in grain may be caused by the differential expression level of genes involve in these processes. Our previous study found significant genotypic differences in grain Cd, Pb, Cr and Cu accumulation among 158 rice varieties grown in 12 locations (Cao et al., 2014). However, the relationship between grain HM accumulation and leaf and stem HM

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Table 1
Means (n = 4) of the biomass and yield components of the two rice genotypes in the different treatments.

Treatment	**Plant height (cm)**		**Spike length (cm)**		**1000-grain weight (g)**		**Yield (g spike^{-1})**	
	Xiushui817	Zheda821	Xiushui817	Zheda821	Xiushui817	Zheda821	Xiushui817	Zheda821
Pb (0)	62.0a	63.3a	10.2a	10.9a	23.6a	25.7a	1.87a	1.38a
Pb (25)	58.9b	61.8b	9.6b	10.3a	21.2b	24.8a	1.31b	1.22b
Pb (50)	60.0b	61.7b	10.0ab	10.6a	21.1b	24.6a	1.39b	1.19b
F-value	6.7**	5.5**	3.8*	2.7	16.6**	3.3	56.4**	10.7**
Cd (0)	60.4a	62.1ab	10.1a	11.0a	22.8a	25.8a	1.79a	1.46a
Cd (5)	60.0a	61.5b	9.9a	10.2b	21.6b	24.7b	1.48b	1.16b
Cd (10)	60.4a	63.0a	9.8a	10.6ab	21.4b	24.8b	1.30c	1.17b
F-value	0.1	3.7*	1.2	4.6*	4.7*	3.7*	38.8**	28.7**
Cr (0)	58.4b	60.5b	10.0a	10.5a	22.5a	25.9a	1.63a	1.29a
Cr (25)	61.1a	62.7a	9.7a	10.7a	21.7a	25.1a	1.41b	1.39a
Cr (50)	61.3a	63.5a	10.1a	10.5a	21.7a	25.2a	1.52ab	1.13b
F-value	7.4**	15.1**	2.1	0.5	1.7	2.4	7.4**	16.9**
Cu (0)	62.4a	63.4a	10.0a	10.5a	22.4a	25.1a	1.51ab	1.28a
Cu (50)	59.8b	61.4b	9.8a	10.6a	22.1ab	25.6a	1.61a	1.27a
Cu (100)	58.6b	62.0b	9.9a	10.6a	21.3b	25.5a	1.45b	1.24a
F-value	10.3**	7.1**	0.4	0.2	2.5	0.8a	3.7*	0.35

Different letters indicate significant differences among the different soil Pb, Cd, Cr and Cu levels ($P < 0.05$). * and ** indicate significance at the 0.05 and 0.01 probability levels, respectively.

concentration at different growth stages in rice has been rarely reported. Many studies have reported on the uptake and translocation of single HMs in barley and rice (Wu et al., 2003; Cao et al., 2015). However, HM pollution in natural environments often occurs due to a combination of different elements. Hence, the current study was carried out to investigate: (1) the effect of combined stress of Cd, Pb, Cr and Cu on biomass and yield components of two rice genotypes differing in grain HM accumulation; (2) the difference in grain HM concentration and its relationship with HM content in stems and leaves at different growth stages; (3) the critical levels of leaves and stems HM concentrations for safe food production.

2. Material and methods

2.1. Experimental design

A pot experiment was conducted in the greenhouse of the Zijingang Campus, Zhejiang University, Hangzhou, China. Two *Japonica* unwaxy rice (*Oryza sativa* L.) genotypes, i.e. Xiushui817 (relatively low grain Cd accumulation) and Zheda821 (high accumulation) were used (Cao et al., 2014). The soil was collected from experimental farm land (0–150 mm in depth) of Zijingang Campus, air-dried and ground to pass through a 2 mm stainless-steel sieve. 6.5 kg soil was weighed and used to fill each pot. The description of soil properties is given in Table S1. The soil pH was measured in water with a 1:2.5 (w/v) soil:distilled water ratio. Ethylene diaminetetra acetic acid (EDTA)-extractable heavy metal concentrations were measured by flame atomic absorption spectrometry (FAAS, SHIMADZU AA-6300, Kyoto, Japan) (Cao et al., 2014). The experiment was designed according to orthogonal design with 4 factors and 3 levels ($L_9 3^4$). The 4 factors were Pb (as $Pb(NO_3)_2$), Cd (as $CdCl_2$), Cr (as $K_2Cr_2O_7$) and Cu (as $CuSO_4$). The 3 different levels of each HM were as follows: 0, 25 and 50 mg kg^{-1} of soil for Pb; 0, 5 and 10 mg kg^{-1} for Cd; 0, 25 and 50 mg kg^{-1} for Cr; 0, 50 and 100 mg kg^{-1} for Cu. According to the orthogonal design, there were nine treatments and each treatment had 4 replicates (Table S2). The soil was artificially contaminated with metal solutions and mixed thoroughly: firstly, Pb was added and then Cd, Cr and finally Cu with an interval of 7 days, respectively. After all the metals were added, the soil was allowed to equilibrate for 30 days in the greenhouse.

2.2. Plant growth, sampling and heavy metal concentration

The seeds of Xiushui817 and Zheda821 were surface sterilized with 2% H_2O_2 for 20 min, then fully rinsed with deionized water, soaked in deionized water at room temperature for 2 days and subsequently germinated for 1 day at 35 °C. Germinated seeds were then sown in sterilized sand and kept in an incubator at 30 °C/26 °C day/night cycle and at 85% relative humidity. At the four-leaf stage, uniform healthy plants were transplanted into the pots. Plants were sampled at seedling, elongation, filling and maturity stages; the plants were thoroughly washed with tap water followed by deionized water at sampling. The plants were then separated into leaves, stems, roots and grains, and dried at 80 °C to constant weight. Different plant organs were powdered and weighted, then ashed at 500 °C for 10 h. The ash was digested with 30% HNO_3 and diluted to a final volume of 25 ml using deionized water. Heavy metal concentrations were measured by flame atomic absorbtion spectroscopy (FAAS). At maturity, plant height, spike length, 1000-grain weight and yield were measured.

2.3. Statistical analysis

Statistical analyses were performed with Data Processing System (DPS) statistical software. $L_9 3^4$ of orthogonal design in DPS were used to evaluate the differences among treatments at significant level of $P < 0.05$ and 0.01. Orthogonal design is used to analysis the comparative effectiveness of several intervention components (Zurovac and Brown, 2012). It can identify the findings more quickly with relative few settings of trials than typical randomized controlled experiments. Regression analysis was carried out to determine the correlations between grain Cd concentration and leaf/stem Cd concentration. According to the kernel maximum permitted contents (MPC) (0.2 mg kg^{-1} DW) and regression equations obtained in the study, leaf- and stem-Cd critical levels at different growth stages for safe rice production were obtained.

3. Results

3.1. Effect of different treatments on plant height, spike length, 1000-grain weight and yield

In the present study, addition of Pb significantly reduced plant height and yield in both genotypes, and 1000-grain weight in Xiushui817 (Table 1). In comparison with Pb(0), yields were decreased by 30% and 26% in XIushui817, and 12% and 14% in Zheda821 by the Pb(25) and Pb(50) treatments, respectively. 1000-grain weight and yield were also decreased in both genotypes by the Cd treatments.