ISSN : 0974 - 7435 Volume 10 Issue 24

2014

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 10(24), 2014 [15610-15618]

Subcellular distribution of lead in different rice cultivars and its relation with lead translocation and grain accumulation

Jianguo Liu[1]*, Wen Zhang[1], Jiakuan Xu[2], Mingxin Wang[1]

[1]School of Environmental and Safety Engineering, Changzhou University, Changzhou 213164, (CHINA)

[2]Changzhou Station for Horticultural Technology Spread, Changzhou 213001, (CHINA)

Email: liujianguo@cczu.edu.cn

ABSTRACT

To test the hypothesis that lead (Pb) content of rice grain may be related to its transport and subcellular distribution in rice plant, the present study was conducted with six rice cultivars of different types under different soil Pb levels. The results showed that grain Pb concentrations were correlated positively and significantly (P<0.05 or 0.01) with distribution ratios (DR) of Pb from shoots to ears/grains, but insignificantly (P>0.05) with the DR from roots to shoots. The DR from shoots to ears/grains were correlated positively and significantly (P<0.05, or 0.01) with subcellular distribution ratios (SDR) of Pb in soluble fraction of shoots, but negatively and significantly (P<0.05, or 0.01) with the SDR in cell wall fraction of shoots. In conclusion, Pb transportation from shoot to grain was the key factor in determining Pb content of rice grain. The Pb distributed in soluble fraction of shoot tissue was the key source of Pb for transferring into the grain. The Pb precipitated in cell wall fraction was the key sink of Pb in shoot tissue for restricting the transport of Pb from shoot to the grain.

KEYWORDS

Lead (Pb); Rice (*Oryza sativa* L.); Cultivars; Type; Subcellular distribution.

INTRODUCTION

Lead (Pb) is one of the most widespread toxic metals. Pb pollution in soils has become increasingly serious in many areas around the world, resulting mainly from gasoline and road traffic, mining and smelting activities, disposal of municipal sewage sludge, paints, and paper pulp[1,2]. In paddy soils around a Pb/Zn mine in China, average Pb concentration of 1486 mg kg^{-1} was reported. Mean Pb concentrations of 419 mg kg^{-1}, 69.1 mg kg^{-1}, 13.2 mg kg^{-1} and 4.67 mg kg^{-1} were found in rice root, straw, grain and brown rice respectively[3]. In Baoji of China, Pb concentrations of some urban soils even exceeded 25000 mg kg^{-1}[4].

Pb is toxic to many organ systems of human body, such as the central and peripheral nervous system, the circulatory system, the excretory system, the cardiovascular systems, and the reproductive system. It can decrease sperm counts and increase prevalence of morphologically abnormal sperm in male, and increase risk of miscarriage in female. Prenatal and early postnatal exposure to Pb at the level 10–20 mg dl^{-1} (in blood) will result in damage to central nervous systems. The damage was characterized by diminished intelligence, shortened attention span, and slowed reaction time. The effects are irreversible, untreatable, and lifelong[5]. Therefore, the absorption and transport of Pb by crops, and its accumulation in edible parts are of great concern. It was reported that heavy metal contamination (Cd and Pb) in food crops grown around mine posed a great health risk to the local population through consumption of rice and vegetables[6].

Genotypic variations within a plant species in the uptake, distribution and accumulation of heavy metals have been observed in rice[7], wheat[8], vegetables[9] and soybeans[10]. A 23.8-fold variation among 138 genotypes of rice in grain Pb concentrations was reported in a slightly contaminated soil[11]. Those results suggested the possibility to develop rice cultivars with low Pb level in the grains. However, the mechanisms on the differences among rice cultivars and types in Pb uptake and translocation are poorly understood.

Subcellular distribution of heavy metals in plant tissues and its relations with phytotoxicology have attracted great interest recently, and it may play an important role in the transfer of metals in plants[12]. To our knowledge, few works have been conducted to study the relationship between subcellular distribution of Pb and its translocation in rice plant and accumulation in the grain. Paddy rice is one of the most important crops in the world, especially in Asia. With the hypothesis that the differences among rice cultivars in subcellular distribution of Pb may play important roles in Pb translocation and accumulation in rice plants, six rice cultivars of different types with variable Pb accumulation abilities[13], were used in the present research. The aim was to investigate whether subcellular distribution of Pb could be a factor, which at least partially determines the differences among rice cultivars in plant Pb translocation and grain Pb accumulation. The results would be valuable for selecting and breeding rice cultivars in order to reduce or prevent Pb contamination in rice.

EXPERIMENTAL

Soil preparation

The soil for the experiment was collected from uncontaminated fields (0–20 cm). After air-dried and passed through a 2-mm sieve, the soil samples were measured for following properties with different experimental techniques: particle size with hydrometer method, pH with pH meter (soil : distilled water = 1 : 2), organic matter with sequential extraction method, cation exchange capacity with ammonium acetate method, and Pb concentration with AAS following H_2O_2–HF–HNO_3–$HClO_4$ digestion[14]. The soil properties are shown in TABLE 1.

Four kilograms of soil was placed in a pot (18 cm in diameter and 20 cm in height). Pb in the form of $PbCl_2$ was added to the soil to obtain Pb levels of 500 mg kg^{-1} (moderately polluted soil) and 1000 mg kg^{-1} (heavily polluted soil) (dry weight). The soil without additional Pb served as control. $PbCl_2$ was dissolved in deionized water and slowly poured into the soil while the soil was mixed manually. The thoroughly mixed soils were placed in the pots and submerged in water (2–3 cm above the soil surface) for a month before rice seedlings were transplanted.

TABLE 1: Selected properties of the soil used in this experiment

Soil type	Soil texture	Particle size (g kg^{-1})			pH	OM [a] (g kg^{-1})	CEC [b] (cmol kg^{-1})	Total Pb (mg kg^{-1})
		Sand	Silt	Clay				
Paddy soil	Sandy loam	565.7 ± 17.8	238.3 ± 4.9	196.0 ± 4.2	6.6 ± 0.3	27.3 ± 0.6	13.9 ± 0.4	37.4 ± 4.1

a Organic matter. *b* Cation exchange capacity. The values are mean ± SE (n = 3).

Rice plant materials

Six rice cultivars varying largely in grain Pb levels were used in this experiment[13]. The cultivars were Liangyoupeijiu (C01, *Hybrid Indica*) and Shanyou 63 (C02, *Hybrid Indica*), high Cd accumulators; CV6 (C03, *Indica*) and Yangdao 6 (C04, *Indica*), moderate Cd accumulators; Wuyunjing 7 (C05, *Japonica*) and Yu 44 (C06, *Japonica*), low Cd accumulators. Rice seeds were submerged in a water bath for about 48 h at room temperature (20–25℃) and germinated under moist condition at 32℃ for another 30 h and the germinated seeds were grown in uncontaminated soil. After 30 days, uniform seedlings were selected and transplanted into the pots (3 seedlings per pot). The pot soil was maintained under flooded condition (2–3 cm of water above soil surface) during the rice growth period.

Experimental design

The experiments were carried out under open-air condition. The pots were arranged in a randomized complete block design with six replicates. The fertilizers of 230 mg of N, 171 mg of K and 68 mg of P were applied to each pot at the following three time points, three days before seedling transplant, and twenty days and seventy days after the transplant.

Determination of Pb concentrations in rice plants

Whole rice plants were sampled at tillering stage, panicle heading stage and at maturity. The rice plants were washed thoroughly with tap water and then with deionized water. The plants were divided into roots, shoots, and ears (at panicle heading stages) or grains (at maturity), and the plant parts were oven-dried at 70°C to a constant weight. The oven-dried samples were ground with a stainless steel grinder to pass through a 100-mesh sieve. Pb concentrations were determined with AAS following HNO_3–$HClO_4$ (4:1, v/v) digestion procedures[15]. For quality control, ten repeated measurements were carried out first with standard Pb solution provided by the Institute of Geophysical and Geochemical Exploration, China. Reagent blanks and certified plant reference material (GBW07602, GSV–3) (provided by the National Research Center for CRM's, China) were run simultaneously with the samples.

Subcellular fractionation and Pb analysis

Whole rice plants were sampled on the 40th day after seedling transplant and subjected to subcellular fractionation and Pb analysis according to Lozano-Rodríguez et al. [16] and Wu et al.[17]

Root and shoot tissues were weighed and immediately frozen in liquid N_2. The frozen shoot and root tissues were homogenized in pre-cold (4°C) extraction buffer [50 mM Tris–HCl, 250 mM sucrose, 1.0 mM DTE, 5.0 mM ascorbic acid and 1.0% (w/v) Polyclar AT PVPP, pH 7.5] with a chilled mortar and a pestle. The homogenate was sieved through a nylon cloth (240 μm), and the residue was designated as cell wall fraction (F1). The filtrate was centrifuged at 10,000 × g for 30 min, and the pellet retained was the organelle-rich fraction (F2). The supernatant was then centrifuged at 100,000 × g for 30 min. The pellet was designated as the membrane-containing fraction (F3), and the supernatant as the soluble fraction (F4). The resultant pellets were re-suspended in extraction buffer. All steps were performed at 4°C.

Pb concentrations in the re-suspended F2 and F3, and in F4 were determined directly with AAS. F1 (cell wall fraction) was dried at 70°C to constant weight, and then digested with acid mixture of HNO_3:$HClO_4$ (2:1, v:v) at 145°C for 24 h. The Pb concentration of the digest was measured with AAS.

Statistical analysis

Data were analyzed with the statistical package SPSS 13.0 and EXCEL 2003 for Windows. Means were compared through one-way ANOVA using Tukey's test at $P<0.05$. Pearson correlations

were used to test the relationships between different parameters, and two significant levels of $P<0.05$ and 0.01 were used in presenting the results.

Distribution ratios (DR) of Pb were calculated with the following equations:

DR of Pb from root to shoot = (Pb accumulation in the aboveground parts / Pb accumulation in the whole plant) × 100%.

DR of Pb from shoot to ear (heading stage) or grain (maturity) = (Pb accumulation in the ear (grain) / Pb accumulation in the aboveground parts) × 100%.

Subcellular distribution ratios (SDR) of Pb were calculated by following:

SDR of Pb in a subcellular fraction = [Pb accumulation of the fraction / total Pb accumulation of the tissue (root or shoot)] × 100%.

RESULTS AND DISCUSSION

Variations among rice cultivars in grain Pb concentrations and Pb distributions and the relations between them

There were significant differences ($P<0.05$) among the rice cultivars in grain Pb concentrations, and the magnitude of variations increased with the rise of soil Pb levels (Figure 1). The grain Pb concentrations of the six cultivars were 0.27−0.49 mg kg^{-1} (1.8-fold variation) for the control, 2.14−5.13 mg kg^{-1} (2.4-fold variation) for 500 mg kg^{-1} soil Pb treatment, and 2.69−7.48 mg kg^{-1} (2.8-fold variation) for 1000 mg kg^{-1} soil Pb treatment.

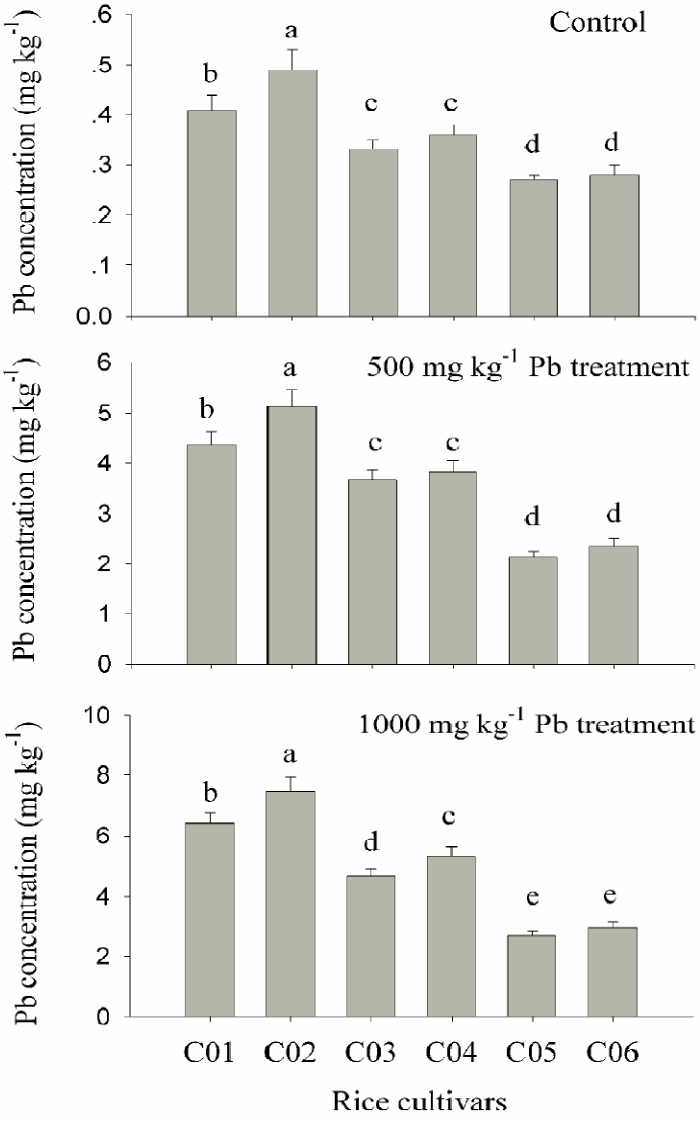

Figure 1: Grain Pb concentrations of different rice cultivars at maturity

The values are mean ± SE (n = 4). Different letters indicate significant differences (P<0.05) between the cultivars.

The distribution ratios (DR) of Pb from roots to shoots varied with soil Pb levels, rice cultivars and rice growth stages (TABLE 2). The DR decreased with the rise of soil Pb levels, and the DR of the control was several times' higher than the DR of soil Pb treatments. The DR at panicle heading and maturity stages was higher than that at tillering stage. There were significant (P<0.05) differences among the six rice cultivars in the DR, but the variations were generally small (from 1.1-fold to 1.6-fold, mostly less than 1.3-fold, the highest / the lowest).

TABLE 2: Distribution ratios (DR) of Pb from roots to shoots (%)

Rice cultivars	Tillering stage			Panicle heading stage			Maturity		
	Control	Pb 500	Pb 1000	Control	Pb 500	Pb 1000	Control	Pb 500	Pb 1000
C01 [a]	33.5 ± 1.2ab	5.6 ± 0.3a	3.6 ± 0.1bc	39.9 ± 1.1bc	7.1 ± 0.1d	5.2 ± 0.2cd	39.0 ± 1.1c	6.9 ± 0.2b	5.6 ± 0.1b
C02	36.4 ± 1.5a	5.4 ± 0.1ab	4.3 ± 0.2a	44.5 ± 2.3a	7.7 ± 0.1b	6.8 ± 0.2a	45.4 ± 1.8a	7.2 ± 02ab	5.8 ± 0.1ab
C03	31.2 ± 0.9bc	5.7 ± 0.2a	4.2 ± 0.1a	42.6 ± 1.6ab	7.8 ± 0.2ab	7.0 ± 0.4a	44.0 ± 1.2ab	7.3 ± 0.1ab	6.0 ± 0.2a
C04	29.5 ± 1.1c	5.5 ± 0.1a	3.7 ± 0.2b	38.2 ± 1.4bc	8.1 ± 0.2a	6.0 ± 0.3b	40.7 ± 1.3bc	7.6 ± 0.3a	5.7 ± 0.2ab
C05	22.1 ± 0.6d	5.4 ± 0.2ab	3.5 ± 0.1bc	36.9 ± 0.8cd	7.5 ± 0.2bc	4.8 ± 0.2d	38.4 ± 1.2c	7.1 ± 0.2ab	4.7 ± 0.1c
C06	29.6 ± 1.0c	5.0 ± 0.1b	3.3 ± 0.1c	33.3 ± 1.2d	7.3 ± 0.1cd	5.5 ± 0.3bc	37.3 ± 0.8c	6.9 ± 0.1b	4.7 ± 0.2c
Average	30.4	5.4	3.8	39.2	7.6	5.9	40.8	7.2	5.4

a C01: Liangyoupeijiu, C02: Shanyou 63, C03: CV6, C04: Yangdao 6, C05: Wuyunjing 7, C06: Yu 44.
The values are mean ± SE (n = 3). Different letters in the same column indicate significant difference between the rice cultivars at P<0.05.

TABLE 3: Distribution ratios (DR) of Pb from shoots to ears (heading stage) or grains (maturity) (%)

Rice cultivars	Panicle heading stage			Maturity		
	Control	Pb 500	Pb 1000	Control	Pb 500	Pb 1000
C01	4.9 ± 0.1a	4.1 ± 0.1a	5.1 ± 0.2a	6.1 ± 0.2a	7.0 ± 0.2b	6.3 ± 0.3b
C02	5.1 ± 0.2a	4.4 ± 0.2a	5.5 ± 0.3a	6.5 ± 0.4a	7.6 ± 0.3a	8.7 ± 0.5a
C03	3.6 ± 0.2c	4.1 ± 0.2a	4.0 ± 0.2b	3.3 ± 0.1d	5.0 ± 0.2d	4.8 ± 0.2c
C04	4.4 ± 0.2b	3.5 ± 0.1b	4.1 ± 0.3b	5.2 ± 0.2b	5.9 ± 0.1c	6.5 ± 0.4b
C05	2.8 ± 0.1d	3.1 ± 0.1c	2.5 ± 0.1c	4.2 ± 0.2c	4.7 ± 0.2de	4.9 ± 0.2c
C06	2.7 ± 0.1d	3.0 ± 0.1c	3.1 ± 0.2c	4.9 ± 0.3b	4.4 ± 0.1e	5.1 ± 0.3c
Average	3.9	3.7	4.1	5.0	5.8	6.1

The values are mean ± SE (n = 3). Different letters in the same column indicate significant difference between the rice cultivars at P <0.05.

The DR from shoots to ears (heading stage) or grains (maturity) were generally small (mostly less than 5%), and were larger at maturity than at panicle heading stage (TABLE 3). There were significant (P<0.05) differences among the rice cultivars in the DR, and the magnitude of variations (from 1.5-fold to 2.2-fold) were larger than those for the DR from roots to shoots.

The correlations between the DR from shoots to ears/grains and grain Pb concentrations were all positive and significant (P<0.01, or 0.05) (TABLE 4). However, the correlations between the DR from roots to shoots and grain Pb concentrations were generally insignificant (P>0.05).

The results indicated that Pb transfer from shoot to grain was a key factor in determining Pb content of rice grain. However, Pb transportation from root to shoot might have little effect on grain Pb content.

TABLE 4: Correlation between distribution ratios (DR) of Pb in rice plants and grain Pb concentrations

Rice growth stages	DR of Pb from roots to shoots			DR of Pb from shoots to ears/grains		
	Control	Pb 500	Pb 1000	Control	Pb 500	Pb 1000
Tillering stage	0.8535*	0.5051	0.6485			
Heading stage	0.7731	0.1990	0.5203	0.9426**	0.9132*	0.9859**
Maturity	0.6665	0.2679	0.7805	0.7681	0.9265**	0.8590*
Average	0.7644	0.3240	0.6498	0.8554*	0.9199**	0.9225**

Significant at $P<0.01$ (**) or 0.05 (*), n = 6.

Variations among rice cultivars in Pb subcellular distributions and its relations with Pb translocations in plants

Subcellular distribution ratios (SDR) of Pb in different fractions of root and shoot tissues differed with soil Pb levels and rice cultivars (TABLE 5). Generally, Pb was mainly stored in cell wall fraction (F1) (about 80% in root tissues and about 70% in shoot tissues). The SDR of Pb in organelle-rich (F2), membrane-containing (F3) and soluble fractions (F4) were generally less than 10% each in root tissues, and about 10% each in shoot tissues.

In root tissues, the SDR in F2 and F3 increased with the rise of soil Pb levels, and the average variations between the control and 1000 mg kg^{-1} soil Pb treatment were 2.2-fold and 2.1-fold, respectively. The SDR in F1 decreased slightly with the increase of soil Pb levels, with less than 1.1-fold variation between the control and 1000 mg kg^{-1} Pb treatment. However, the SDR in F4 fell obviously with the elevating of soil Pb levels, and the average variation was 2.6-fold. In shoot tissues, the SDR in F1 increased with the increase of soil Pb levels, and the average variation between the control and 1000 mg kg^{-1} soil Pb treatment was 1.1-fold. The SDR in F4 decreased sharply with the rise of soil Pb levels, with 2.8-fold variation among the soil Pb levels. The SDR in F2 and F3 changed little among the soil Pb levels.

TABLE 5: Subcellular distribution ratios (SDR) of Pb in the roots and shoots of different rice cultivars

Soil Pb treatment (mg kg^{-1})	Rice cultivars	Pb distribution ratio in roots (%)				Pb distribution ratio in shoots (%)			
		F1 [a]	F2	F3	F4	F1	F2	F3	F4
Control	C01	82.1 ± 0.9ab	3.8 ± 0.1bc	4.3 ± 0.1ab	9.7 ± 0.5b	62.4 ± 0.4b	10.6 ± 0.1d	10.4 ± 0.2bc	16.6 ± 0.4a
	C02	80.9 ± 0.6b	3.4 ± 0.1c	4.2 ± 0.1b	11.5 ± 0.6a	61.7 ± 0.5b	11.3 ± 0.2bc	10.0 ± 0.2c	17.0 ± 0.6a
	C03	83.0 ± 0.8ab	3.5 ± 0.2c	4.6 ± 0.2a	8.9 ± 0.3c	65.3 ± 0.7a	12.4 ± 0.3a	10.8 ± 0.3b	11.5 ± 0.3c
	C04	82.6 ± 1.0ab	4.3 ± 0.3a	4.6 ± 0.2a	8.5 ± 0.4cd	64.8 ± 0.6a	10.8 ± 0.2cd	11.6 ± 0.2a	12.8 ± 0.5b
	C05	83.8 ± 0.9a	4.1 ± 0.2ab	4.3 ± 0.2ab	7.7 ± 0.3de	66.8 ± 1.1a	11.6 ± 0.3b	11.8 ± 0.4a	9.8 ± 0.2d
	C06	84.4 ± 1.2a	4.0 ± 0.2ab	4.2 ± 0.2b	7.5 ± 0.4e	66.0 ± 0.8a	12.3 ± 0.2a	11.5 ± 0.3a	10.2 ± 0.4d
	Average	82.8	3.8	4.4	9.0	64.5	11.5	11.0	13.0
500	C01	80.4 ± 0.8b	7.3 ± 0.2b	7.7 ± 0.2b	4.5 ± 0.2c	67.1 ± 0.7c	11.7 ± 0.2a	10.7 ± 0.2a	10.5 ± 0.2b
	C02	77.4 ± 0.7c	8.2 ± 0.3a	9.4 ± 0.4a	5.0 ± 0.3c	66.7 ± 0.5c	12.1 ± 0.3a	9.5 ± 0.3cd	11.6 ± 0.3a
	C03	77.2 ± 0.5c	7.9 ± 0.3a	8.7 ± 0.3a	6.2 ± 0.3b	70.5 ± 0.6b	11.0 ± 0.2b	9.9 ± 0.1bc	8.6 ± 0.2c
	C04	81.2 ± 0.7ab	4.7 ± 0.3d	6.0 ± 0.2c	8.1 ± 0.3a	72.0 ± 0.8ab	10.4 ± 0.1c	8.9 ± 0.1d	8.8 ± 0.1c
	C05	83.3 ± 1.2a	4.9 ± 0.1d	5.7 ± 0.3c	6.0 ± 0.2b	74.3 ± 1.5a	11.2 ± 0.2b	9.3 ± 0.2cd	5.2 ± 0.2d
	C06	82.4 ± 1.0ab	6.6 ± 0.2c	7.3 ± 0.3b	3.7 ± 0.1d	73.8 ± 1.1a	11.1 ± 0.3b	10.3 ± 0.3ab	4.8 ± 0.1d
	Average	80.3	6.6	7.5	5.6	70.7	11.3	9.8	8.2
1000	C01	78.6 ± 0.8bc	8.7 ± 0.3b	10.2 ± 0.3b	2.5 ± 0.3c	70.6 ± 0.6cd	12.6 ± 0.3a	11.0 ± 0.2b	5.8 ± 0.2b
	C02	76.6 ± 0.6c	8.5 ± 0.2b	9.2 ± 0.2c	5.7 ± 0.3a	67.6 ± 0.7d	12.6 ± 0.2a	12.2 ± 0.3a	7.6 ± 0.2a
	C03	74.4 ± 0.7c	10.7 ± 0.4a	11.2 ± 0.5a	3.8 ± 0.2b	74.8 ± 1.1ab	10.1 ± 0.1c	10.8 ± 0.2b	4.3 ± 0.1c
	C04	80.2 ± 0.8ab	7.2 ± 0.2c	8.3 ± 0.1d	4.2 ± 0.1b	72.8 ± 0.8bc	12.8 ± 0.3a	9.9 ± 0.3c	4.5 ± 0.2c
	C05	82.8 ± 1.6a	7.1 ± 0.1c	7.9 ± 0.2d	2.2 ± 0.2c	77.6 ± 1.7a	10.4 ± 0.2bc	9.6 ± 0.1c	2.5 ± 0.2d
	C06	82.1 ± 1.1a	7.3 ± 0.2c	8.4 ± 0.2cd	2.2 ± 0.1c	76.7 ± 1.0a	10.8 ± 0.2b	9.6 ± 0.2c	2.8 ± 0.1d
	Average	79.1	8.3	9.2	3.4	73.3	11.5	10.5	4.6

a F1: cell wall fraction, F2: organelle-rich fraction, F3: membrane-containing fraction, F4: soluble fraction. The values are mean ± SE (n = 3). Different letters in the same column indicate significant difference between the rice cultivars at $P<0.05$.

The variations of the SDR among the rice cultivars were large in F4, and the magnitude of the variations increased with the elevation of soil Pb levels. The variations ranged from 1.5-fold to 2.6-fold in root tissues, and from 1.7-fold to 3.1-fold in shoot tissues. The variations in F2 and F3 ranged from 1.1-fold to 1.7-fold in root and shoot tissues, but the variations were not related to soil Pb levels. The variations were relatively small in F1 (about 1.1-fold variation in roots and shoots).

Many researches have tried to link subcellular distributions of heavy metals to their detoxifications in the plants[18-20]. Immobilization of Pb in cell wall was considered as one of tolerance mechanisms in *Typha latifolia*[21]. However, in *Thlaspi caerulescens,* Cd and Zn were found mainly in vacuole[22], and the sequestration of the metals in cytosol was regarded as an important mechanism for metal tolerance and accumulation in the plant[23].

Subcellular distributions of heavy metals may vary among plant species, because *Tetraselmis suecica* and *Skeletonema costatum* accumulated a high proportion of Cu (>90%) in insoluble fractions, while *Haslea ostrearia* incorporated a greater proportion of Cu (>75%) in cytosolic fraction[24]. In *Thlaspi goesingense, Alyssum bertolonii* and *Alyssum lesbiacum*, Ni was mainly stored in the vacuoles of epidermis cells[25], while in *Berkheya coddii*, higher Ni concentrations were found in the cuticle of epidermis[26]. It was also reported that As was mainly stored in the cytoplasm, while Cu was mostly in the cell wall[27]. Therefore, the response of plants to heavy metals were also metal-dependent[28].

TABLE 6: Correlation between subcellular distribution ratios (SDR) of Pb and distribution ratios (DR) of Pb in rice plants

Soil Pb treatment (mg kg^{-1})	Rice growth stages	Between SDR of Pb in roots and DR of Pb from roots to shoots				Between SDR of Pb in shoots and DR of Pb from shoots to ears/grains			
		F1	F2	F3	F4	F1	F2	F3	F4
Control	Tillering	-0.7624	-0.6853	-0.1091	0.8203*				
	Heading	-0.8501*	-0.7620	0.2185	0.8678*	-0.9372**	-0.7314	-07558	0.9575**
	Maturity	-0.7360	-0.7739	0.2596	0.7690	-0.8153*	-0.6883	-0.5501	0.8207*
500	Tillering	-0.5507	0.2601	0.2238	0.4482				
	Heading	-0.3571	-0.2178	-0.0404	0.8379*	-0.9294**	0.5515	0.1991	0.9266**
	Maturity	-0.3408	-0.2540	-0.0809	0.8841*	-0.9210**	0.6233	0.0625	0.9339**
1000	Tillering	-0.8741*	0.6963	0.5487	0.8551*				
	Heading	-0.8513*	0.6818	0.5326	0.8311*	-0.9686**	0.7585	0.8813*	0.9667**
	Maturity	-0.8989*	0.7380	0.7269	0.7098	-0.9252**	0.7969	0.7894	0.9046*

Significant at $P<0.01$ (**) or 0.05 (*), n = 6.

Correlation analysis showed that the DR of Pb from shoots to ears/grains were negatively and significantly ($P<0.01$, or 0.05) correlated with the SDR of Pb in F1 of shoots (TABLE 6). The correlations between the DR from roots to shoots and the SDR in F1 of roots were also negative but only partially significant ($P<0.05$).

The results suggested that cell wall fraction of Pb was the key Pb sink in shoot tissue for restricting Pb transport from shoot to the grain, and cell wall fraction of Pb in root tissue may be one of the important factors that influence Pb translocation from root to shoot.

Inconsistency and generally insignificance ($P>0.05$) were found in the correlations between the DR from roots to shoots and the SDR in F2 and F3 of roots, and between the DR from shoots to ears/grains and the SDR in F2 and F3 of shoots.

The DR from shoots to ears/grains were positively and significantly ($P<0.01$, or 0.05) correlated with the SDR in F4 of shoots. The correlations between the DR from roots to shoots and the SDR in F4 of roots were also positive and mostly significant ($P<0.05$).

The results demonstrated that the Pb distributed in soluble fraction of shoot tissue was the key source of Pb for translocating into the grain. The Pb in soluble fraction of root tissue may be one of the main Pb sources for the transportation from root to shoot.

Former studies on Cd subcellular distribution and translocation were conducted with two rice cultivars in solution cultivations, and the results indicated that larger ratios of Cd in soluble fraction in the roots were associated with higher ratios of Cd distribution from the roots to the shoots[29,30]. Our results on the relation between Pb subcellular distribution in root and its translocation from root to shoot were generally in agreement with their results on Cd.

Variations of grain Pb concentrations, Pb translocations and subcellular distributions among rice types

Grain Pb concentrations of the rice types were in the order of *Hybrid Indica* > *Indica* > *Japonica*, and the differences between the types were all significant (P<0.05). The DR from shoots to ear/grain was generally in the order of *Hybrid Indica* > *Indica* > *Japonica*, and the differences between the types were mostly significant (P<0.05). The SDR in F4 of shoots was also in the order of *Hybrid Indica* > *Indica* > *Japonica*, and the differences between the types were generally significant (P<0.05). However, the present and former studies showed that significant differences (P<0.05) also existed among the rice cultivars within the same rice types in grain Pb contents, the DR and the SDR (Liu et al., 2003). Therefore, whether rice type is an important factor affecting Pb translocation in rice plant and accumulation in the grain need further studies.

The results of this study would be helpful for the understanding on the mechanisms of Pb uptake, transfer and accumulation in rice plant.

CONCLUSION

The rice cultivars that varied largely in grain Pb contents differed greatly in distribution ratios (DR) of Pb from shoots to ears/grains, and in subcellular distribution ratios (SDR) of Pb in soluble fraction (F4). The transfer of Pb from shoot to grain was a key factor in determining grain Pb contents that was little affected by the Pb transportation from root to shoot. The Pb in soluble fraction of shoot tissue is the key source of Pb that would be transported into rice grain. The Pb in soluble fraction of root tissue may be one of the main Pb sources for the translocation from root to shoot. Pb was mainly stored in cell wall fraction of shoot (about 70%) and root (about 80%). The Pb precipitated in cell wall was the key sink of Pb in rice shoot, which restricted Pb transport from shoot to the grain. Pb sequestration in cell wall of root may be one of the important factors which influence Pb translocation from root to shoot. With regard to the hypothesis proposed, subcellular distribution of Pb in rice plants may be one of the main mechanisms that regulates Pb translocation and differentiates rice cultivars in the level of Pb accumulation in the grains.

ACKNOWLEDGMENTS

This research was financially supported by the National Natural Science Foundation of China (31071350, 70901035).

REFERENCES

[1] S.K.Yadav; South Afr.J.Bot., **76**, 167 **(2010)**.
[2] T.T.T.Duong, B.K.Lee; J.Environ.Manage., **92**, 554 **(2011)**.
[3] Q.W.Yang, W.S.Shu, J.W.Qiu, H.B.Wang, C.Y.Lan; Environ.Int., **30**, 883 **(2004)**.
[4] B.Wei, L.Yang; Microchem.J., **94**, 99 **(2010)**.
[5] E.K.Silbergeld, M.Waalkes, J.M.Rice; Am.J.Ind.Med., **38**, 316 **(2000)**.
[6] P.Zhuang, M.B.McBride, H.P.Xia, N.Y.Li, Z.Li; Sci.Total Environ., **407**, 1551 **(2009)**.
[7] J.Shi, L.Q.Li, G.X.Pan; J.Environ.Sci., **21**, 168 **(2009)**.
[8] V.Page, U.Feller; Ann.Bot., **96**, 425 **(2005)**.
[9] P.D.Alexander, B.J.Alloway, A.M.Dourado; Environ.Pollut., **144**, 736 **(2006)**.
[10] T.Arao, N.Ae; Plant Soil, **251**, 247 **(2003)**.

[11] F.R.Zeng, Y.Mao, W.D.Cheng, F.B.Wu, G.P.Zhang; Environ.Pollut., **153**, 309 **(2008)**.
[12] A.Geffard, H.Sartelet, J.Garric, S.Biagianti-Risbourg, L.Delahaut, O.Geffard; Chemosphere, **78**, 822 **(2010)**.
[13] J.G.Liu, K.Q.Li, J.K.Xu, Z.J.Zhang, T.B.Ma, X.L.Lu, J.C.Yang, Q.S.Zhu; Plant Sci., **165**, 793 **(2003)**.
[14] M.C.Amacher; Nickel, cadmium, and lead, in D.L.Sparks, Covering ed., 'Methods of Soil Analysis, part 3-Chemical Methods', Soil Science Society of America, Inc. and American Society of Agronomy, Inc., Madison (WI), 739–768 **(1996)**.
[15] S.E.Allen; Analysis of vegetation and other organic materials, in S.E.Allen, Covering ed., 'Chemical Analysis of Ecological Materials', Blackwell Scientific Publications, Oxford, 46–61 **(1989)**.
[16] E.Lozano-Rodríguez, L.E.Hernández, P.Bonay, R.O.Carpena-Ruiz; J.Exp.Bot., **48**, 123 **(1997)**.
[17] F.B.Wu, J.Dong, Y.Cai, F.Chen, G.P.Zhang; Sci.Total Environ., **385**, 228 **(2007)**.
[18] M.J.Wang, W.X.Wang; Envion.Sci.Technol., **42**, 940 **(2008)**.
[19] W.X.Wang, R.Guan; Environ.Toxicol.Chem., **29**, 1841 **(2010)**.
[20] Y.Wu, W.X.Wang; Environ.Pollut., **159**, 3097 **(2011)**.
[21] L.Lyubenova, P.Pongrac, K.Vogel-Mikus, G.K.Mezek, P.Vavpetic, N.Grlj, P.Kump, M.Necemer, M.Regvar, P.Pelicon, P.Schroder; Metallomics, **4**, 333 **(2012)**.
[22] J.F.Ma, D.Ueno, F.J.Zhao, S.P.McGrath; Planta, **220**, 731 **(2005)**.
[23] T.B.Chen, X.L.Yan, X.Y.Liao, X.Y.Xiao, Z.C.Huang, H.Xie, L.M.Qu; Chin.Sci.Bull., **50**, 2739 **(2005)**.
[24] C.Amiard-Triquet, B.Berthet, L.Joux, H.Perrein-Ettajani; Environ.Toxicol., **21**, 1 **(2006)**.
[25] H.Küpper, E.Lombi, F.J.Zhao, G.Wieshammer, S.P.McGrath; J.Exp.Bot., **52**, 2291 **(2001)**.
[26] B.H.Robinson, E.Lombi, F.J.Zhao, S.P.McGrath; New Phytol., **158**, 279 **(2003)**.
[27] Y.Q.Zheng, X.J.Dai, L.Wang, W.Z.Xu, Z.Y.He, M.Ma; J.Plant Physiol., **165**, 1906 **(2008)**.
[28] A.J.Miao, W.X.Wang; Aquat.Toxicol., **78**, 114 **(2006)**.
[29] J.Y.He, C.Zhu, Y.F.Ren, Y.P.Yan, C.Cheng, D.A.Jiang, Z.X.Sun; Pedosphere, **18**, 371 **(2008)**.
[30] H.Yu, Z.X.Xiang, Y.Zhu, J.L.Wang, Z.J.Yang, Z.Y.Yang; J.Plant Nutr., **35**, 71 **(2012)**.