Ecotoxicology and Environmental Safety 90 (2013) 35–40

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

# Genotypic differences among rice cultivars in lead accumulation and translocation and the relation with grain Pb levels

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ARTICLE INFO

*Article history:*
Received 5 November 2012
Received in revised form
8 December 2012
Accepted 10 December 2012
Available online 9 January 2013

*Keywords:*
Rice (*Oryza sativa* L.)
Lead
Grain
Genotype
Uptake
Translocation

ABSTRACT

In order to understand the differences among rice cultivars and genotypes in lead (Pb) uptake and translocation, and their relationship with Pb accumulation in rice grains, pot soil experiments were carried out with six rice cultivars of diverse types under different soil Pb levels. The results showed that the differences among rice cultivars in Pb concentrations varied largely with plant organs, and the magnitudes of the differences were larger in ears and grains than in shoots and roots. Pb concentrations in ears and grains differed significantly ($p < 0.05$) between rice types, and were in the order: *Hybrid Indica* > *Indica* > *Japonica*. Grain Pb concentrations were correlated significantly ($p < 0.05$) with shoot Pb concentrations, and highly significantly ($p < 0.01$) with ear Pb concentrations, but generally not with root Pb concentrations. The differences among rice cultivars in translocation factors (TF) of Pb from shoots to ears/grains were generally larger than the TF of Pb from roots to shoots. The differences among rice types in TF of Pb from shoots to ears/grains were generally significant ($p < 0.01$ or 0.05), and the TF were in the order *Hybrid Indica* > *Indica* > *Japonica*. But the differences between rice types in the TF of Pb from roots to shoots were mostly insignificant ($p > 0.05$). In general, grain Pb concentrations were correlated significantly ($p < 0.01$ or 0.05) with the TF of Pb from shoots to ears/grains, but insignificantly ($p > 0.05$) with the TF of Pb from roots to shoots. So the Pb in shoots, but not in roots, may be the main sources of Pb transferred to the grains. Pb concentrations in rice grains are likely to be determined mainly by the translocations of Pb from shoots to the grains, and little by the transport from roots to shoots. Pb concentration in ears of heading can be used as an index of Pb level in the grains.



## 1. Introduction

Lead (Pb) is a widespread contaminant in the environment, and highly toxic to biological systems. Owing to industrial development and population expansion, Pb pollution has become increasingly serious in many areas around the world, and Pb concentrations in agricultural soil also increased rapidly in China (Li et al., 2000). The main source of Pb pollution is metal smelting (Capdevila et al., 2003; Caussy et al., 2003), but the Pb from agriculture, industry, and urban activities are also very important (Marchiol et al., 2004). In paddy soils around a lead/zinc (Pb/Zn) mine in Lechang of Guangdong Province of China, average Pb concentration of 1486 mg $kg^{-1}$ was reported. Mean Pb concentrations of 419 mg $kg^{-1}$, 69.1 mg $kg^{-1}$, 44.9 mg $kg^{-1}$, 21.9 mg $kg^{-1}$, 13.2 mg $kg^{-1}$ and 4.67 mg $kg^{-1}$ were found in rice root, straw, stalk, hull, grain and brown rice respectively (Yang et al., 2004).

Of all the metals, Pb is toxic to many organ systems of human body, such as the central and peripheral nervous system, the red blood cells, the kidneys, the cardiovascular systems, and the male and female reproduction organs. It is currently considered by the International Agency for Research on Cancer (IARC) to be a possible human carcinogen (group 2B; Silbergeld et al., 2000). So Pb absorption and transport in plants are of public concern, especially about its translocation to edible parts. It was reported that the rice and vegetables growing in Pb-contaminated areas can pose a great health risk to the local population (Zhuang et al., 2009). Some researches showed that the uptake, transport and accumulation of Pb by plants are strongly governed by plant factors, and they differed significantly with plant species, and Pb accumulation and distribution also differed largely among plant organs (Zhang et al., 1998; Yang et al., 2004; Yoon et al., 2006). But the relations between plant organs in Pb uptake and distribution are not clear.

Paddy rice is one of the most important crops in the world, especially in Asia. It was reported that there were significant genotypic differences in heavy metal concentrations of rice grains under slightly contaminated soil (Zeng et al., 2008). The results

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0147-6513/$ - see front matter 
http://dx.doi.org/10.1016/j.ecoenv.2012.12.007

suggest the possibility to develop the rice cultivars with low Pb concentrations in the grains. However, the differences among rice cultivars and genotypes in Pb uptake and translocation in considerable and heavy Pb-contaminated soils are seldom reported.

Based on our previous studies (Liu et al., 2003), six rice cultivars of different genotypes with variable grain Pb levels while growing in Pb-contaminated soil were used in the present experiment. The aims of the study were to investigate: 1) the variations among rice cultivars and types in Pb concentrations in different parts of rice plants; 2) the variations among rice cultivars and types in Pb translocation factors (TF) from roots to shoots and from shoots to ears/grains; and 3) the relationships between grain Pb concentrations and Pb concentrations in other parts of rice plants, and the TF of Pb between the parts. The information will be useful in the selection and breeding of rice cultivars to reduce Pb in the diet in Pb-contaminated areas.

## 2. Materials and methods

### 2.1. Soil preparation and analysis

The soil for the experiment was collected from uncontaminated fields (0–20 cm). After air-drying and passing through a 2-mm sieve, the following soil properties were tested: (1) particle size with hydrometer method; (2) pH with a pH meter (soil:distilled water=1:2); (3) organic matter with sequential extraction method; (4) cation exchange capacity with ammonium acetate method; and (5) Pb concentration with AAS following $H_2O_2$–HF–$HNO_3$–$HClO_4$ digestion (Sparks, 1996). The properties are shown in Table 1, and the data also presented in our previous publication (Liu et al., 2011). The soil is a sandy loam with a high proportion of sand and neutral pH. It contains a moderate level of organic matter, CEC, and a low level of Pb.

Four kilograms soil was placed in each 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 and 1000 mg $kg^{-1}$ (dry weight).

$PbCl_2$ was dissolved in deionized water and poured into the soil slowly while mixing the soil at the same time. The thoroughly mixed soils were stored in pots and submerged in water (2–3 cm above the soil surface) for a month before rice seedlings were transplanted. Soils without adding Pb served as control.

### 2.2. Rice plant materials

Based on our previous studies (Liu et al., 2003), six rice cultivars of different types varying in grain Pb levels were used in this experiment. They were Liangyoupeijiu (C01, *Hybrid Indica*, a high grain Pb accumulator), Shanyou 63 (C02, *Hybrid Indica*, a high grain Pb accumulator), CV6 (C03, *Indica*, a moderate grain Pb accumulator), Yangdao 6 (C04, *Indica*, a moderate grain Pb accumulator), Wuyunjing 7 (C05, *Japonica*, a low grain Pb accumulator) and Yu 44 (C06, *Japonica*, a low grain Pb accumulator). Rice seeds were submerged in a water bath for about 48 h at room temperature (20–25 °C) and germinated under moist condition (seeds were covered with two layers of moist gauze cloth) at 32 °C for another 30 h and the germinated seeds were grown in uncontaminated soils. After 30 days, the uniform seedlings were selected and transplanted into the pots (3 plants per pot). The pot soil was maintained under flooded conditions (with 2–3 cm of water above soil surface) during the rice growth period.

### 2.3. Experimental design

The experiments were carried out under open-air conditions. The pots were arranged in a randomized complete block design with six replicates. 230 mg of N, 171 mg of K and 68 mg of P were applied to each pot at three times: the 3rd day before seedling transplant, and the 20th and 70th day after the transplant.

**Table 1**
Selected properties of the soil used in this experiment ($n=3$).

| Soil type | Soil texture | Particle size (g $kg^{-1}$) | | | pH | OM [a] (mg $kg^{-1}$) | CEC [b] (g $kg^{-1}$) | Total Pb (cmol $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | | Sand | Silt | Clay | | | | |
| Paddy soil | Sandy loam | 587.5 | 233.2 | 179.3 | 6.8 | 27.6 | 13.6 | 36.5 |

[a] Organic matter.
[b] Cation exchange capacity.

### 2.4. 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 root, shoot, ear (at panicle heading stage) and grain (at maturity), and oven-dried at 70 °C to constant weight. The oven-dried samples were ground with a stainless steel grinder to pass through a 100-mesh sieve. Pb concentrations of the samples were determined with AAS following $HNO_3$–$HClO_4$ ($HNO_3$:$HClO_4$=4:1, V:V) digestion procedures (Allen, 1989).

Data were analyzed with the statistical package SPSS 13.0 and EXCEL 2003 for Windows. Two significant levels of $p<0.05$ and 0.01 were used in presenting the results.

## 3. Results

### 3.1. Variations among rice cultivars and types in Pb accumulations and the relation with grain Pb levels

The differences among rice cultivars in Pb concentrations in different parts of rice plants at different growth stages are presented in Tables 2–4.

The variations among different parts of rice plants in Pb concentrations were huge. Pb concentrations were generally in the order root ≫shoot > ear (at heading stage) > grain (at maturity). In general, the magnitudes of the differences increased with the increase of soil Pb levels, and also with the extending of plant growth stages. For example, the average Pb concentration ratios of roots to shoots at tillering stage were 4.8:1, 37.3:1 and 61.8:1 for the control, 500 and 1000 mg $kg^{-1}$ soil Pb treatments, respectively. The corresponding average ratios of roots to shoots to ears at panicle heading stage were 15.5:3.2:1, 135.3:3.6:1 and 176.0:3.0:1, and the ratios of roots to shoots to grains at maturity were 74.5:17.9:1, 560.7:14.9:1 and 775.2:14.5:1. So, Pb concentrations in rice grains were only one tenths of that in shoots and one hundredths of that in roots in considerable and heavy Pb-contaminated soils.

The differences among rice cultivars in Pb concentrations varied largely with the parts of rice plants. The magnitudes of the differences were in the order ear (heading stage) > grain (maturity) ≫ shoot > root. The absolute differences among rice cultivars (the percentage of the highest cultivar being higher than that of the lowest cultivar) ranged from 7.7 to 83.1 percent for roots, from 12.5 to 116 percent for shoots, from 103.6 to 162.3 percent for ears (at panicle heading stage), and from 89.7 to 109.7 percent for grains (at maturity). The average differences were

**Table 2**
Pb concentrations in different parts of different rice cultivars at tillering stage (mg $kg^{-1}$).

| Rice cultivars | Control | | Pb500[a] | | Pb1000[b] | |
|---|---|---|---|---|---|---|
| | Root | Shoot | Root | Shoot | Root | Shoot |
| C01 | 26.52 | 6.56 | 1887.06 | 55.45 | 3581.69 | 83.93 |
| C02 | 30.89 | 8.49 | 2248.33 | 59.47 | 6558.16 | 93.88 |
| C03 | 28.45 | 5.79 | 2074.35 | 49.45 | 5331.74 | 76.22 |
| C04 | 29.38 | 6.10 | 1704.68 | 55.18 | 5272.06 | 84.25 |
| C05 | 28.92 | 3.93 | 1756.72 | 45.58 | 4588.42 | 79.93 |
| C06 | 27.22 | 5.09 | 1648.68 | 38.46 | 4366.92 | 62.65 |
| Average | 28.56 | 5.99 | 1886.64 | 50.60 | 4949.83 | 80.14 |
| $LSD_{0.05}$ | 2.46 | 0.38 | 217.35 | 3.19 | 482.81 | 2.96 |

Same for all following tables.
[a] Soil Pb treatments of 500 mg $kg^{-1}$.
[b] Soil Pb treatments of 1000 mg $kg^{-1}$.