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# Field experiment for determining lead accumulation in rice grains of different genotypes and correlation with iron oxides deposited on rhizosphere soil



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### HIGHLIGHTS

- The available Pb in rhizosphere soil would be reduced significantly by planting paddy rice cultivars.
- High-Pb accumulation rice cultivars can absorb Pb in grains exceeding 0.2 mg  $\rm kg^{-1}$  from a soil with Pb above 60 mg  $\rm kg^{-1}$ .
- Iron oxides deposited on rhizosphere soil rather than on rice root surface dominate the sequestration of soil available Pb.
- Less Pb accumulation in brown rice would be caused by higher tendency of Pb sequestration in rhizosphere soil.
- Enhancement of iron oxide deposits on rhizosphere soil will result in reduction of Pb accumulation in brown rice.

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### GRAPHICAL ABSTRACT



### ABSTRACT

Paddy rice (*Oryza sativa* L.) is a major staple crop in Asia. However, heavy metal accumulation in paddy soil poses a health risk for rice consumption. Although plant uptake of Pb is usually low, Pb concentrations in rice plants have been increasing with Pb contamination in paddy fields. It is known that iron oxide deposits in the rhizo-sphere influence the absorption of soil Pb by rice plants. In this study, 14 rice cultivars bred in Taiwan, including ten japonica cultivars (HL21, KH145, TC192, TK9, TK14, TK16, TN11, TNG71, TNG84, and TY3) and four indica cultivars (TCS10, TCS17, TCSW2, and TNGS22), were used in a field experiment. We investigated the genotypic variation in rice plant Pb in relation to iron oxides deposited in the rhizosphere, as seen in a suspiciously contaminated site in central Taiwan. The results showed that the cultivars TCSW2, TN11, TNG71, and TNG84 accumulated brown rice Pb exceeding the tolerable level of 0.2 mg kg<sup>-1</sup>. In contrast, the cultivars TNG522, TK9, TK14, and TY3 accumulated much lower brown rice Pb (<0.1 mg kg<sup>-1</sup>); therefore, they should be prioritized as safe cultivars for sites with potential contamination. Moreover, the iron oxides deposited on the rhizosphere soil show stronger affinity to soil-available Pb than those on the root surface to form iron plaque. The relative tendency of Pb sequestration toward rhizosphere soil was negatively correlated with the Pb concentrations in brown rice. The iron oxides deposited on the rhizosphere soil but not on the root surface to form iron plaque dominate

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Pb sequestration in the rhizosphere. Therefore, the enhancement of iron oxide deposits on the rhizosphere soil could serve as a barrier preventing soil Pb on the root surface and result in reduced Pb accumulation in brown rice.

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### 1. Introduction

Lead (Pb) has been accumulating in soil owing to anthropogenic activities such as mining, smelting of Pb ores, burning of coal, effluents from storage battery industries, automobile exhausts, deposition of shots and sinkers, and use of sewage sludge (Dudka and Adriano, 1997; Lofts et al., 2007; Navarro et al., 2008; Fu et al., 2008; WHO, 2011). Typical mean Pb concentration for surface soils worldwide averages 32 mg kg<sup>-1</sup> and ranges from 10 to 67 mg kg<sup>-1</sup>; soil Pb concentrations over 100 mg kg $^{-1}$  would represent potentially contaminated soils (Kabata-Pendias and Pendias, 2001). There is a great disparity between countries as to the maximum acceptable concentrations of metals established by them for agricultural soils receiving anthropogenic inputs of metals (Khodaverdiloo et al., 2011). The maximum acceptable concentrations of Pb among different countries are in the ranges of 50–300 mg kg<sup>-1</sup> (McLaughlin et al., 2000). Generally, it has been considered safe to use garden produce grown in soils with total lead levels <300 mg kg<sup>-1</sup> (Wuana and Okieimen, 2011). With increased Pb levels in soil, plants would be showing increased Pb uptake (Rooney et al., 1999; Jarvis and Leung, 2002; Wu et al., 2011). The risk of lead poisoning through the food chain thus will increase as the soil Pb level rises above the concentration criteria (i.e.  $300 \text{ mg kg}^{-1}$ ).

Recently, Pb toxicity in humans owing to the consumption of Pbcontaminated rice has become an issue of increasing concern (Fu et al., 2008; Chamannejadian et al., 2013; Naseri et al., 2015). The Joint FAO/WHO Expert Committee on Food Additives (WHO, 1993) recommended a provisional tolerable weekly intake (PTWI) of 25  $\mu$ g Pb kg<sup>-1</sup> body weight for humans (Fang et al., 2014). In a large-scale survey of rice grains from markets and fields by Norton et al. (2014), only 0.6% of the samples exceeded the common safety threshold of 0.2 mg Pb kg $^{-1}$  for rice grains (CFSA, 2005; FAO/WHO, 2011). Nevertheless, rice has been identified as one of the major sources of Pb intake in some Asian countries (Shimbo et al., 2001; Zhang et al., 1998; Moon et al., 1995). Rice samples with Pb exceeding 0.2 mg kg<sup>-1</sup> usually came from Asian countries such as Bangladesh, China, and India. In China, the high Pb concentrations exceeding safety standards in rice grains were usually attributable to local anthropogenic sources (Zhang and Ke, 2004; Cheng et al., 2006; Fu et al., 2008). Williams et al. (2009) reported that in southeast China, rice grown near or in industrial areas could accumulate high Pb concentrations. Fang et al. (2014) reported that the average concentration of Pb in rice samples from the different growing regions in China was about 0.10 mg kg $^{-1}$ ; they suggested that the Pb background levels of the rice grown in southern and southwest China would be above 0.06 mg  $kg^{-1}$  and higher than those in the other regions of China. In northern Iran, a preliminary investigation of a dataset with 60 samples showed that the average Pb in rice grains at the time of harvesting was 2.23 mg kg<sup>-1</sup> (Khaniki and Zazoli, 2005). Another survey in Khuzestan, Iran, showed that the mean of Pb concentrations in rice grains exceeded 0.4 mg kg<sup>-1</sup> in the rice cultivars Anburi and Champa; this was attributable to air and soil pollution, topographical and physiological conditions in the cultivation region, and uncontrolled use of organophosphorous fertilizers (Ramezani et al., 2011). In central Taiwan, harvested rice grains showed Pb accumulation exceeding 0.2 mg kg<sup>-1</sup> (Li et al., 2016); these rice plants were grown in Wuxi river basin soil with Pb level < 300 mg kg<sup>-1</sup>, which is the threshold for declaring soil Pb contamination in Taiwan (EPA-TW, 2011). In China, Fu et al. (2008) noted that the average Pb concentration in rice grains would be one to several times higher than the maximum allowable concentration (i.e., 0.2 mg kg<sup>-1</sup>) in slightly Pb-contaminated areas where the soil Pb level is <100 mg kg<sup>-1</sup>. The high ability of rice plant to take up soil Pb and accumulate it in grain could be interpreted by integrating the dynamic factors, which are related the physiological and ecological mechanisms governing metal uptake and transfer in plant (Baltrenaite et al., 2012).

Lead uptake by rice plants is generally related to Pb availability in paddy soil (Ok et al., 2011); this will be a conditional function of the soil-available Pb, rice genotype, and soil characteristics (Li et al., 2016). To reduce Pb accumulation in rice grains, Pb availability in soil and the different Pb uptake and translocation capabilities of rice genotypes should be considered. Either by increasing sorption or precipitation, application of certain soil amendments may decrease the mobility and availability of heavy metals (Lee et al., 2009). Especially the organic-material amendments can not only reduce metal availability but also generally improve soil fertility (Clemente et al., 2006; Park et al., 2011; Juang et al., 2012). Organic amendments have been found to be promising for the stabilization of soil heavy metals in situ (Basta and McGowen, 2004; Brown et al., 2004). Recently, Zheng et al. (2015) conducted a field experiment in Hunan, China for the study on mitigating heavy metal accumulation into rice using biochar amendment. Immobilization of Pb in paddy soils and reduction of Pb uptake by rice due to biochar application have been explored in the previous studies (Ashraf et al., 2015). Otherwise, many studies highlighted genotypic differences among rice cultivars in grain Pb concentrations. Liu et al. (2003) conducted a pot experiment with soil Pb concentration of 800 mg kg<sup>-1</sup> and grain Pb concentration of 2.7–4.8 mg kg<sup>-1</sup> for 20 rice cultivars. In other studies (Liu et al., 2013; Liu et al., 2015), pot experiments were conducted with 6 rice cultivars grown in soils with Pb spikes of 500 and 1000 mg kg<sup>-1</sup>. The results showed that grain Pb concentrations were 2.39–7.53 mg  $kg^{-1}$  with the soil Pb spikes and 0.29–0.55 mg kg<sup>-1</sup> in the control with soil Pb concentration < 40 mg kg<sup>-1</sup>. Cheng et al. (2006) conducted a large-scale field survey with 12 japonica rice cultivars grown at three ecologically different locations; they found that the average grain Pb concentration was 0.1135 mg kg<sup>-1</sup>, and ~15% of all samples exceeded the allowable level (i.e., 0.2 mg  $kg^{-1}$ ).

It has recently been reported that iron plague on the root surface could sequester heavy metals and therefore influence heavy metal uptake by rice plants (Liu et al., 2008; Lei et al., 2011; Syu et al., 2013). Zheng et al. (2012) suggested that enhanced iron plaque formation significantly sequestered Pb and reduced the Pb concentration in rice shoots. Nevertheless, there were significant differences in iron plaque formation among different rice genotypes (Lee et al., 2013). Liu et al. (2011) reported the influence of iron plaque formation on plant Pb uptake with different rice cultivars; they suggested that japonica type shows a genetic ability to form superior iron plaques, and it would have lower Pb uptake compared to indica type. Li et al. (2016) recently reported that Pb uptake by rice plants in relation to iron plaque formation was simultaneously influenced by soil characteristics and rice genotypes. In addition, the difference in iron plaque formation among rice cultivars showed a strong relation with radial oxygen loss (ROL) (Mei et al., 2009; Wang et al., 2011). ROL from roots into the rhizosphere created an oxidizing environment; this is essential for oxidization from ferrous (Fe<sup>2+</sup>) to ferric (Fe<sup>3+</sup>), and it leads to a welldefined zone of ferric (hydro-)oxide accumulation (Mendellsohn et al., 1995; Armstrong and Armstrong, 2005). Thus, the ferric (hydro-)oxides that deposit on the root surface, that is, the iron plaque, can sequester heavy metals on the root surfaces. However, ROL measurements vary greatly under different experimental conditions

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