Ecotoxicology and Environmental Safety 128 (2016) 206–212

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Interaction between sulfur and lead in toxicity, iron plaque formation and lead accumulation in rice plant

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ARTICLE INFO

Article history:
Received 3 August 2015
Received in revised form
18 February 2016
Accepted 19 February 2016
Available online 4 March 2016

Keywords:
Glutathione
Iron plaque
Lead
Rice (*Oryza sativa* L.)
Sulfur

ABSTRACT

Human activities have resulted in lead and sulfur accumulation in paddy soils in parts of southern China. A combined soil–sand pot experiment was conducted to investigate the influence of S supply on iron plaque formation and Pb accumulation in rice (*Oryza sativa* L.) under two Pb levels (0 and 600 mg kg^{-1}), combined with four S concentrations (0, 30, 60, and 120 mg kg^{-1}). Results showed that S supply significantly decreased Pb accumulation in straw and grains of rice. This result may be attributed to the enhancement of Fe plaque formation, decrease of Pb availability in soil, and increase of reduced glutathione (GSH) in rice leaves. Moderate S supply (30 mg kg^{-1}) significantly increased Fe plaque formation on the root surface and in the rhizosphere, whereas excessive S supply (60 and 120 mg kg^{-1}) significantly decreased the amounts of iron plaque on the root surface. Sulfur supply significantly enhanced the GSH contents in leaves of rice plants under Pb treatment. With excessive S application, the rice root acted as a more effective barrier to Pb accumulation compared with iron plaque. Excessive S supply may result in a higher monosulfide toxicity and decreased iron plaque formation on the root surface during flooded conditions. However, excessive S supply could effectively decrease Pb availability in soils and reduce Pb accumulation in rice plants.

1. Introduction

Lead levels in paddy soils of China have been significantly enhanced recently by anthropogenic activities, such as mining, atmospheric deposition or irrigation with contaminated groundwater (Ye et al., 2004; Luo et al., 2011). Rice grown on Pb-contaminated paddy soil can accumulate high levels of Pb in shoots and grains (Zhuang et al., 2009; Liu et al., 2013). The Pb accumulated in rice plants can enter the food chain, leading to serious health risks to the human body, particularly the central nervous system of children (Wasserman et al., 1997). Thus, preventing Pb uptake and translocation in rice grown in Pb-contaminated soils is important (McLaughlin et al., 1999).

The increasing sulfur accumulation in paddy soils in southern China was confirmed by agricultural activities, such as anthropogenic SO_2 emission, super-phosphate fertilization, and wastewater irrigation (Hu and Xu, 2002; Wang et al., 2004). Sulfur plays an important role in regulating plant growth and development (Anjum et al., 2008). However, more S accumulation in the paddy soils may disturb the uptake of other elements (e.g., As and Cd) by rice, as S can be easily affected by the change of redox potential (Eh) of paddy soils because of the transformation of the redox state of inorganic species from −2 to +6 (Hu and Xu, 2002; Hu et al., 2007; Fan et al., 2010).

The oxidizing capacity, which is characterized as radial oxygen loss (ROL), of paddy rice roots can lead to the formation of iron oxyhydroxide plaque on the root surface (Mei et al., 2012; Cheng et al., 2014; Yang et al., 2016). The effect of Fe plaque on the uptake of metals (e.g., Zn) depends on the amount of Fe plaque on the root surfaces (Otte et al., 1989; Zhang et al., 1998; Yang et al., 2014). The enhancement of Fe plaque formation was demonstrated to reduce the accumulation of As, Cd, and Pb in grains of rice (Hu et al., 2007; Cheng et al., 2014). Fe plaque can act as a barrier or buffer to the uptake of heavy metals probably because of the adsorption and immobilization of metals on plaque (Taylor and Crowder 1983; Liu et al., 2004; Mei et al., 2012; Yang et al., 2016).

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http://dx.doi.org/10.1016/j.ecoenv.2016.02.021
0147-6513/

Sulfur may alleviate the influence of heavy metal on plant growth by S metabolism. Reduced glutathione (GSH), which is a low molecular weight tri-peptide, detoxifies heavy metals through the formation of phytochelatins (PCs), which can synthesize metal–PC complex and transport the complex to the vacuole (Sun et al., 2005; Khan et al., 2008; Gupta et al., 2013). In addition, S metabolism is closely linked to the biosynthesis of PCs in plants and metal sequestration (Thangavel et al., 2007). Recent experimental studies showed that S application could protect plants from heavy metal (e.g., As and Cd) toxicity because of the increase of GSH contents in the leaves of the plants (Hu et al., 2007; Fan et al., 2010, 2013; Zhang et al., 2013). It is necessary to clarify the interaction between S and Pb in toxicity, iron plaque formation and Pb accumulation in rice plant. In order to evaluate variation of S supply and Fe plaque on Pb accumulation and the distribution in rice plants, the major aim of the present study was to investigate the variations and correlations in GSH contents, degrees of Fe plaque formation, and the uptake and distribution of Pb in shoot and root tissues and Fe plaque on root surfaces and in the rhizospheres under moderate and excessive S conditions.

2. Materials and methods

2.1. Soil used and experimental design

The soil used in the pot trial was collected from a hydragric paddy field (0–20 cm) located at Soil Fertility and Fertilizer Efficiency Long Term Monitoring Base of Qiyang City, Hunan Province, China. The soil was thoroughly mixed, air-dried, and ground to <2 mm. The physical and chemical properties of the soil were analyzed and presented as follows: pH: 5.31; organic matter: 22.6 g kg^{-1}; total N: 1.65 g kg^{-1}; total S: 191 mg kg^{-1}; available S: 13.45 mg kg^{-1}; total Pb: 41.99 mg kg^{-1}; available Pb: 7.32 mg kg^{-1}; available Fe: 128.7 mg kg^{-1}; and available Mn: 25.4 mg kg^{-1}.

A rhizobag system with a soil–sand combination was used to collect rhizosphere and non-rhizosphere soils separately and study the effect of S application on iron plaque formation, rice leaf GSH, and Pb uptake in rice plants. Six treatments were employed with two levels of Pb (as $PbCl_2$) [without Pb (Pb0), 600 mg Pb kg^{-1} (Pb600)], combined with four levels of S (as Na_2SO_4) [0 (S0), 30 (S30), 60 (S60), 120 (S120) mg S kg^{-1})]. In total, 1.5 kg of dried soil was placed in each pot. The soil used was firstly spiked with Pb (0 and 600 mg kg^{-1} supplied as $PbCl_2$), then mixed thoroughly and allowed to equilibrate for 2 months. After 2 months, the bulk soil was air-dried and passed through a 2-mm sieve, and then the air-dried Pb polluted-soil was spiked with S (0 30, 60 and 120 mg kg^{-1} supplied as Na_2SO_4) and equilibrated for 1 month. After 1 month, the bulk soil was also air-dried, passed through a 2-mm sieve and then received (per kg) a basal application of 100 mg P as KH_2PO_4; 125 mg K as KH_2PO_4; and 110 mg N as urea. During the equilibration, the soil water was maintained at 70% of maximum water holding capacity by weight. Rhizobags, which were made of nylon netting with a mesh size of 40 μm, were 4 cm in diameter and 10 cm height and filled with 0.3 kg of quartz sand. The quartz sand was collected from Jinwuxing market, Beijing, PR China, and was not contaminated with heavy metals. Before use, the quartz sand was deionized water-washed, air-dried, and sieved (<2 mm). The sand-filled rhizobags were placed in the center of each soil pot (15 cm diameter $\times$ 17 cm height). The sand-filled rhizobags were placed in the center of each soil pot. This rhizobag design could successfully prevent the roots and root hairs from entering the adjacent non-rhizosphere soil zone while allowing the transfer of microfauna and root exudates between the two compartments. This outcome meant that although the soil was used to enclose the outside portion of the rhizobag, the rhizosphere was confined to the sand compartment and effectively separated from the non-rhizosphere soil compartment (Hu et al., 2007; Yang et al., 2014, 2016). The plants were transplanted into sand at the beginning of the pot experiment, and the sand is here referred to as “rhizosphere” material and at the end of the study and the soil outside of the rhizobag is referred to as “non-rhizosphere” (the comparable soil zone). The rest of the pot outside the rhizobag was filled with 1.5 kg of air-dried soil. Each treatment was repeated four times. The soils were balanced at 100% of the water holding capacity for two weeks before planting.

The rice seeds of cultivar no. 12 Zhe-You were sterilized in 10% H_2O_2 (v/v) solution for 30 min and thoroughly washed with deionized water. The seeds were germinated in the moist filter paper at a temperature of 26 °C. In each pot, two uniform germinated seeds were transplanted into each rhizobag and grown for 120 days. Soil moisture content was increased to 100% of the water holding capacity before seedling emergence and kept submerged in deionized water for the whole growth period. All the pots were arranged randomly in the greenhouse with a relative humidity of 85% and light/dark cycle of 14 h day/10 h night.

2.2. Harvest and sampling

Before harvesting, the plant height was measured, and two pieces of fresh penultimate leaves of each plant were sampled, wrapped round with aluminum foils, and stored in liquid nitrogen for GSH analysis. The rice plants were harvested by carefully removing the rhizobags from the pots. The roots were separated from the quartz sand. The plants were divided into three parts as roots, straw, and grains. All the plants were rinsed with deionized water. The straw and grains were dried to a constant weight at 60 °C for 48 h, and the dry weights were recorded. The fresh roots were used for the extraction of iron plaque. The quartz sand inside the rhizobags was referred to as the rhizosphere material. The soils in the pots were homogenized thoroughly and further referred to as the non-rhizosphere soils (Hu et al., 2007).

2.3. Extraction of iron plaque

During harvest, one of fresh root in the rhizobag was used for Pb, Fe, and Mn on the root surface by a dithionitecitrate-bicarbonate (DCB) method (Taylor and Crowder, 1983), and the other fresh root was used for the S on the root surface by 1 M HCl solution (Hu et al., 2007). The quartz sand that was collected from the rhizosphere was extracted by 0.1 M HCl solution for Pb, Fe, Mn, and S analysis (Hu et al., 2007). The rice roots or quartz sand were immersed in 45 mL DCB or HCl solution, respectively, and shaken at 280 rpm for 3 h at 25 °C. The solution was filtered with quantitative filter papers to 100 mL volumetric flasks, rinsed four times, and diluted to the volume with deionized water. After the extraction with DCB or HCl solution, the roots were oven dried to constant weight at 60 °C for 48 h, and the dry weights were recorded. The dry weights of roots, straw, and grains for each plant were referred to as plant biomass.

2.4. Plant digestions

The oven-dried root, straw, and grain samples were homogenized using a Retsch grinder (Type: 2 mm, Retsch Company, Germany) and digested by HNO_3 (guaranteed reagent) (Alexander et al., 2006). The sub-samples of plant tissues (0.5 g) were digested in flasks on an electric heating plate at 60 °C and then increased to 110 °C and kept stable until the sample solution became clear. The sample volume was adjusted to 50 mL with ultrapure water. Blank and reference material (BGW-07603) (China Standard Materials Research Center, Beijing, PR China) were utilized for quality control. The Pb recovery rates were $90 \pm 10\%$.

2.5. Chemical analysis

The Pb, Fe, and Mn concentrations in the DCB extracts and HCl