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Cadmium uptake, accumulation, and remobilization in iron plaque and rice tissues at different growth stages

Hang Zhou, Wei Zhu, Wen-Tao Yang, Jiao-Feng Gu, Zi-Xiang Gao, Li-Wei Chen, Wen-Qi Du, Ping Zhang, Pei-Qin Peng, Bo-Han Liao*

College of Environmental Science and Engineering, Central South University of Forestry and Technology, Changsha 410004, China

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ABSTRACT

Rice consumption is considered the main source of human dietary Cd intake in Southeast Asia. This study aimed to investigate Cd uptake, accumulation, and remobilization in iron plaque and rice (*Oryza sativa* L. cv. 'Xiangwanxian 12') tissues at different growth stages. A pot experiment was performed in two Cd-contaminated paddy soils. Cd concentrations in iron plaque and rice tissues at five different growth stages (tillering, booting, milky, dough, and maturing) were measured. Cd concentrations in iron plaque and rice tissues (roots, stems, leaves, spikelet, husks, and brown rice) varied with growth stage. Cd accumulation in rice plants increased with extending growth in both soils, reaching 15.3 and 35.4 μg/pot, respectively, at the maturing stage. The amounts of Cd in brown rice increased from the milky to maturing stages, with the greatest percentage uptake during the maturing stage. Cd amount in iron plaque significantly affected the uptake and accumulation of Cd in roots and aerial parts of rice plants. Accumulated Cd in leaves was remobilized and transported during the booting to maturing stages, and the contributions of Cd transportation from leaves to brown rice were 30.0% and 22.5% in the two soils, respectively. A large amount of Cd accumulated in brown rice during the maturing stage. The transportation of remobilized Cd from leaves was also important for the accumulation of Cd in brown rice.

1. Introduction

Cadmium (Cd) is a highly toxic element and usually is present at very low levels in the soil environment; however, human interventions have caused an increased Cd in soil. When the level of soil Cd exceeds a certain proportion, it can lead to a reduction in yield and quality of agricultural crops (Wei et al., 2010; Rahman et al., 2014). In addition, the Cd that is taken up by roots from the polluted soil and transported to the edible parts of crops has toxic effects on human health through the food chain (Boularbah et al., 2006; Xin et al., 2010; Wang et al., 2011), and chronic exposure to Cd may decrease the viability and MT protein of HepG2 and KERTr cells (Xue et al., 2017). At present, food consumption has been identified as the major pathway for human exposure to Cd (Zhu et al., 2011; Ding et al., 2013; Moreno-Jiménez et al., 2016). It is estimated that in the United States the average person consumes about 30 μg of Cd per day in food, with the largest contribution coming from cereal grain products, potatoes, and other vegetables (Tavarez et al., 2015). Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population (Huang et al., 2011; Ueno et al., 2011). In Southeast Asia (China, Japan, and India, etc.), rice product consumption is considered to be the main source of human dietary intake of Cd (Ueno et al., 2011; Hu et al., 2013; Zhao et al., 2014). Therefore, China has established a strict limit of 0.2 mg/kg for Cd in brown rice (GB 2762-2012).

Iron plaques commonly form on the surfaces of rice root as a result of the release of oxygen and oxidants into the rhizosphere (Liu et al., 2005 and 2006). Fe and Mn are the main elements of iron plaque on the surface of rice roots, and amounts of metals and metalloids such as Cd, Pb, Cu, Zn, As etc. can be sequestered by iron plaque (Liu et al., 2005; Yamaguchi et al., 2014). Some research found the iron plaque on the surface of rice root had a significantly effect on Cd accumulation in rice root, and considered that iron plaques can play an important role in the prevention of Cd transferring from soil to rice root (Liu et al., 2007, 2008, and 2010; Cheng et al., 2014; Zhou et al., 2015).

Rice is readily able to uptake Cd and to transport it to the aerial parts, where it accumulates in the brown rice grain, even when Cd concentrations in the soil are low (Yu et al., 2006; Wang et al., 2011). Some studies have indicated that Cd accumulation in brown rice occurs through root uptake, root-to-shoot translocation, and shoot-to-grain translocation during rice grain development (Cheng et al., 2005; Uraguchi and Fujiwara, 2012; Liu et al., 2014; Zhou et al., 2015). The period from the beginning of the filling stage to the maturing stage is

* Corresponding author.
E-mail address: liaobohan1020@163.com (B.-H. Liao).

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the critical period for nutrient accumulation in grain (Wu et al., 2010), and Cd is also transported into the brown rice during this period (Yang et al., 2009). Harris and Taylor (2013) used a pair of near-isogenic lines (NILs) of durum wheat differing in the accumulation of Cd in the grain and found a direct pathway of Cd transport from roots to grain via xylem-to-phloem transfer in the stem throughout the grain-filling period. Yoneyama et al. (2010) determined Cd concentrations in xylem and phloem saps and in different rice tissues, and found that Cd accumulated in brown rice via xylem loading and transportation in roots, and xylem-to-phloem transfer at the stem and spikelet nodes. Fujimaki et al. (2010), conducted noninvasive studies through the addition of a ^{107}Cd tracer to the hydroponic culture solution, and concluded that the nodes were the central organ where xylem-to-phloem transfer took place at the grain-filling stage. Furthermore, in recent years, some studies reported that the Cd accumulated in actively transpiring parts of the plant such as culms, rachis, flag leaves, and external parts of the panicles could be quickly remobilized and transported via the phloem to the grain (Rodda et al., 2011; Uraguchi and Fujiwara, 2013). Yan et al. (2010) reported that the Cd remobilized to the grain via phloem transportation during leaf senescence accounted for a larger proportion than that directly transported from the root via xylem-phloem transportation under hydroponic conditions. Approximately 60% of the Cd in brown rice grain was remobilized from that accumulated in rice plants before flowering stage (Rodda et al., 2011). These studies indicated that the Cd accumulation in brown rice was not only related to root uptake and root-to-shoot translocation, but also to the redistribution and remobilization of Cd in rice plants during the period of grain-filling to the maturing stage. Hence, Cd accumulation in brown rice was affected by a source-sink relationship during the plant growth stages (Cheng et al., 2005). Although some researchers have investigated Cd uptake, remobilization, and transportation at different rice growth stages in hydroponics experiments, the results from Cd-contaminated soils are unclear.

In this study, a pot experiment of rice cultivation was carried out in two Cd-contaminated paddy soils (Cd concentrations were 0.96 mg/kg and 3.76 mg/kg). The concentrations of Cd in the iron plaque and rice tissues (roots, stems, leaves, spikelet, husks, and brown rice) at five different growth stages (tillering, booting, milky, dough, and maturing) were measured. The main objectives of this study were (i) to investigate Cd uptake, accumulation, and remobilization in iron plaque and rice tissues at different growth stages; (ii) to investigate Cd accumulation in brown rice in relation to root uptake and remobilization in rice plants; and (iii) to quantitatively estimate the contribution of transportation of accumulated Cd from leaves to levels in brown rice.

2. Materials and methods

2.1. Experiment site and soil preparation

The experiment was carried out in the open-air experimental ground of Central South University of Forestry and Technology in Changsha city, Hunan Province, China. The area is in a mid-continental, humid subtropical, monsoon climate zone, with a mean annual temperature of 17.2 °C, an annual accumulated temperature of 5457 °C, a mean annual precipitation in the range of 1361.6 mm, and a frost-free period of about 275 days. The soil samples used were collected from the rice fields close to the Shizhuyuan Mine Zone of Chenzhou city, southern Hunan Province, China. Ploughed soil of 0–20 cm depth was taken and transported to the laboratory. After being air-dried at room temperature, the soil samples were crushed, large debris, stones, and pebbles were removed, and the samples were passed through a 5-mesh sieve. The soil type was Hydragric anthrosols, and pH, organic matter (OM), cation exchange capacity (CEC), and Cd concentration were 6.02, 41.3 g/kg, 10.28 cmol/kg, and 0.96 mg/kg for soil L, and 6.00, 40.4 g/kg, 18.95 cmol/kg, 3.76 mg/kg for soil H, respectively.

2.2. Pot experiments

Using plastic buckets (diameter 20 cm, height 24 cm), 4.5 kg of prepared soil (soil L or H) was submerged with 2–3 cm of water above the soil surface for 20 d. Three days before transplantation of rice seedlings, urea ($CO(NH_2)_2$), ammonium phosphate ($(NH_4)PO_4$), and potassium carbonate (K_2CO_3) were used in all treatments, giving application rates of 0.28 g/kg N, 0.21 g/kg P (P_2O_5), and 0.22 g/kg K (K_2O), respectively.

The conventional rice (*Oryza sativa* L.) cultivar 'Xiangwanxian 12' was chosen in this experiment because it is widely grown in Hunan Province, China. After being surface-sterilized in 30% (v/v) hydrogen peroxide (H_2O_2) solution for 20 min, the rice seeds were thoroughly washed with deionized water, soaked in water for germination at 30 ± 0.5 °C for about 48 h, and then sown in a paddy field with clean soil on June 20, 2014. Rice seedlings with three tillers were transplanted into each pot on July 20, 2014. Fifteen pots each were planted with one of two soils (soil L or H). During the whole growth period, the pot soil was maintained under flood conditions with 3–4 cm of water on top of the soil, except for the soil-drying period of 1 week towards the end of the tillering stage and before harvest. N, P (P_2O_5), and K (K_2O) were also applied at a rate of 0.14 g/kg, 0.105 g/kg, and 0.11 g/kg, respectively, at the early of the booting stage.

Three rice plants were harvested at the end of the tillering stage (60 d after germination), at the booting stage (80 d), at the milky stage (100 d), at the dough stage (113 d), and at the maturing stage (120 d), respectively. The rice plants were cleaned with deionized water, put in a 105 °C drying oven for 30 min, and dried at 70 °C to constant weight. The samples were separated into root, stem, leaf, spikelet, husk, and brown rice. No spikelet, husk, and brown rice subsamples were collected from the rice plants at the tillering stage and the booting stage. The weights of all subsamples of rice plants were recorded, and then the samples were ground to a powder and kept in a cleaned polyethylene container before analysis.

2.3. Iron plaque extraction

Fresh roots harvested at days 60, 80, 100, 113, and 120 were subjected to iron plaque extraction using dithionite–citrate–bicarbonate (DCB) solution containing 0.03 mol/L citrate ($Na_3C_6H_5O_7{\cdot}2H_2O$), 0.125 mol/L bicarbonate ($NaHCO_3$), and 1.0 g of dithionite ($Na_2S_2O_4$) (Otte et al., 1991; Liu et al., 2006). The roots were immersed in 30 mL of DCB reagent at room temperature (20–25 °C) for 60 min until the roots changed in color from brown or reddish-brown to white. The roots were then rinsed three times with deionized water, and DCB solutions were diluted to 100 mL with deionized water. Following extraction, the rice roots lacking iron plaque were then dried in a 70 °C oven to constant weight, and then weighed accurately.

2.4. Chemical analysis and quality control

Soil organic matter (OM) content was determined calorimetrically by oxidation with potassium dichromate (Nelson et al., 1996). Cation exchange capacity (CEC) was determined using the ammonium acetate method after washing with alcohol (Kahr and Madsen, 1995). The roots (without iron plaque), stems, leaves, spikelet, husks, and brown rice samples harvested at days 60, 80, 100, 113, and 120 were digested using the dry ashing method (Hseu, 2004). Concentrations of Fe, Mn, and Cd in the samples were determined with inductively coupled plasma optical emission spectrometry (ICP-AES 6300, Thermofisher, USA) or graphite furnace atomic absorption spectrometry (FAAS, ICE 3500, Thermofisher, USA).

In order to monitor the quality of chemical analyses and to examine the accuracy of data, quality control measures, which included reagent blanks, triplicate samples and certified reference materials, were taken consistently during the course of pretreatment and analysis. Standard