Environmental Pollution 209 (2016) 169–176

Contents lists available at ScienceDirect

Environmental Pollution

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

Uptake, translocation and transformation of antimony in rice (*Oryza sativa* L.) seedlings

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ARTICLE INFO

Article history:
Received 27 September 2015
Received in revised form
16 November 2015
Accepted 20 November 2015
Available online 10 December 2015

Keywords:
Antimony
Rice cultivars
Iron plaque
Translocation
Transformation

ABSTRACT

Antimony (Sb), as a toxic metalloid, has been gaining increasing research concerns due mainly to its severe pollution in many places. Rice has been identified to be the dominant intake route of Sb by residents close to the Sb mining areas. A hydroponic experiment was conducted to investigate the difference in uptake, translocation and transformation of Sb in rice seedlings of four cultivars exposed to 0.2 or 1.0 mg/L of Sb(V). The results showed that mass concentration of iron plaque (mg/kg FW) formed at the root surfaces of cultivar N was the highest among all tested cultivars at both low and high exposure levels of Sb(V). The accumulated Sb concentration in iron plaque significantly increased with an increase in mass concentration of iron plaque formed at the rice root. The total amount of iron plaque (mg/pot) at rice root generally increased with increasing exposed Sb(V) concentration, which was closely associated with the increasing lipid peroxidation in roots. Concentration percentage of Sb in rice root significantly reduced as the corresponding value in the iron plaque increased, suggesting that iron plaque formation strongly suppressed uptake of Sb by rice root. Sb concentration in rice tissues followed an order: root > stem, leaf. The japonica rice (cultivars N and Z) exhibited a stronger translocation tendency of Sb from root to stem than indica hybrid rice (cultivars F and G). Translocation of Sb from root of cultivar F to its stem and leaf was sharply enhanced with increasing Sb exposure concentration. Sb(V) could be reduced to Sb(III) in rice tissues, especially in stems (10–26% of the total Sb). For the sake of food safety, the difference in uptake, translocation and transformation of Sb in rice species planted in Sb-contaminated soils should be taken into consideration.

1. Introduction

Antimony (Sb), as an analog of arsenic (As), has been listed as a priority pollutant by the United States Environmental Protection Agency and the European Union (Cui et al., 2015). Sb has no known biological function, but it is toxic (Filella et al., 2002). It was estimated that the average crustal abundance of Sb is 0.2 mg/kg (Smith and Huyck, 1999). Its environmental concentrations have been increasing due to human activities, such as mining, smelting, fossil fuel combustion and waste incineration. The maximum permissible pollutant concentration of Sb in soil recommended by WHO is 36 mg/kg (Chang et al., 2002). It was reported that high Sb concentrations at some sites far exceeded the standard. In Spain, the Sb concentrations in three abandoned Sb mining areas in Extremadura reached up to 225–2449.8 mg/kg (Murciego et al., 2007). China is a major producer of Sb, and most Sb mines are in Hunan and Guangxi Provinces (Huang et al., 2011). The Sb concentration in paddy soils near Xikuang Mountain Sb mining area in Hunan Province has reached 1565 mg/kg (He and Yang, 1999). Plant grown on Sb contaminated soils can accumulate high levels of Sb (Murciego et al., 2007), posing a great threat to human health through food chain.

Rice, as a stable food for about 3 billion people, has been identified to be a major route for Sb exposure, especially in mining areas of Asian countries (Ren et al., 2014a). Wu et al. (2011b) reported that for the residents close to the Xikuang Mountain Sb mine, 33% of the total daily intake of Sb was from rice, which was higher than other exposure routes. Hence, it is indispensible to study the uptake and translocation of Sb in rice for a better understanding of its health risks to human beings.

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http://dx.doi.org/10.1016/j.envpol.2015.11.033
0269-7491/

As a semi-aquatic plant, rice growing under anaerobic conditions is able to form iron plaques coated on its root surfaces. This is because rice can release oxygen and oxidants to the rhizosphere through developed aerenchyma. The Fe(II) in the rhizosphere is then oxidized to Fe(III) which may precipitate on the root surfaces in the forms of ferric hydroxides, goethite, and lepidocrocite (Liu et al., 2011; Ren et al., 2014a). A significant amount of metal (loid)s including Mn, Zn, Pb, Cu, and As can be associated with iron plaques due to high affinity of iron (hydr)- oxides for them (Tripathi et al., 2014). Hence, iron plaques play an important role in uptake of metal (loid)s by rice. Huang et al. (2011) revealed that 40–80% of total Sb was accumulated in iron plaque at the root surfaces of rice. Ren et al. (2014a) reported that the presence of iron plaque decreased uptake of both Sb(V) and Sb(III) by rice roots during a short-term exposure experiment. Another study also consistently suggested that the presence of iron plaque significantly reduced Sb uptake by rice roots, whereas it did not decrease Sb concentration in rice shoots (Cui et al., 2015). Overall, the possible transformation of Sb in rice tissues was not considered in aforementioned work by Huang et al. (2011) and Cui et al. (2015), and none of these three studies measured the MDA content in their tested rice species. However, some other studies inconsistently showed that iron plaque increased the uptake of both nutrient and toxic elements into plants (Zhang et al., 1998; Ye et al., 2001). These divergent findings demonstrated that roles of iron plaque in uptake of Sb by rice still remain largely unclear, which need to be further addressed.

Previous studies showed that As accumulation in rice tissues varied with different cultivars (Liu et al., 2004a; Hua et al., 2011). Based on the fact that Sb and As belong to the same group of the periodic table of elements, uptake, translocation and transformation of Sb in rice could also be affected by cultivars. Huang et al. (2011) documented that cultivars played an important role in uptake and translocation of different species of Sb in rice. Ren et al. (2014a) used only one cultivar to test the uptake and transformation of Sb, and the authors found that Sb(V) was the predominant form in rice as rice seedlings were exposed to both Sb(III) and Sb(V), but the possible difference in transformation of Sb between different rice species was not taken into consideration.

The toxicity and bioavailability of Sb depend not only on its total concentration, but also on its chemical species. Similar to As, Sb in the environment has both inorganic and organic forms, and the inorganic species are more toxic and abundant than the organic ones (Gebel, 1997). Sb exists in two common inorganic forms, which are antimonite (Sb(V)) and antimonite (Sb(III)). Although Sb(III) is generally more toxic than Sb(V), antimony is mainly present as Sb(V) in soils (Filella et al., 2009). Also, Sb(V) is the prevalent Sb oxidation state in aerobic circumstances (Mitsunobu et al., 2006; Okkenhaug et al., 2012). As stated before, high concentrations of Sb have been detected in many places, but the mechanisms regulating uptake, translocation and transformation of Sb in plants are still unclear (Feng et al., 2013). To achieve this scientific gap, rice seedlings were exposed to Sb(V) using hydroponic experimental systems. The specific objectives were to: 1) evaluate the effects of Sb(V) on development and growth (biomass) of different rice cultivars; 2) investigate the differences in iron plaque formation at rice root surfaces and its role in uptake of Sb(V) by rice; 3) study the translocation and transformation of Sb in different rice cultivars.

2. Materials and methods

2.1. Cultivation of rice

Seeds of four rice (*Oryza sativa* L.) cultivars including Fengliangyou-6(F), Guodao-6(G), Nanjing-45(N), Zhendao-11(Z) were obtained from Jiangsu Academy of Agricultural Sciences. Cultivars F and G belong to indica hybrid rice while cultivar N and Z belong to japonica rice. All the four cultivars are grown in China with large area. Seeds were sterilized in 30% H_2O_2 solution for 15 min and rinsed thoroughly with Milli-Q water. They were then soaked in Milli-Q water for 48 h, and germinated on wet filter papers placed in petri dishes. After germination, uniform seeds were selected and transferred to 96-orfice plates, which floated on solution containing 0.5 mM $Ca(NO_3)_2$.

The nutrient solution used in our experiment is recommended by International Rice Research Institute (Wu et al., 2011a). The full strength nutrient solution contained 2 mg/L of Fe as Fe(II)-ethylenediaminetetraacetic acid (EDTA), 40 mg/L of N as NH_4NO_3, 10 mg/L of P as NaH_2PO_4, 40 mg/L of K as K_2SO_4, 40 mg/L of Ca as $CaCl_2$, 40 mg/L of Mg as $MgSO_4$, and trace elements Mn, Mo, B, Zn and Cu. At one-leaf stage, rice seedlings were grown in 1/4 strength nutrient solution for 2 weeks. At three-leaf stage, 1/2 strength nutrient solution was used for 1 week. After then, the rice seedlings were transferred and cultivated in pots with 550 mL full strength nutrient solution for 1 week. The nutrient solution pH was adjusted to 5.5–5.8 with KOH and renewed twice per week. During the cultivation period, all plants were placed in a greenhouse set as 16 h daytime with light density of 180–240 $\mu mol/(m^2 \cdot s)$, 28 and 20 °C day and night temperatures, and 60–70% relative humidity.

2.2. Sb(V) uptake by rice

After 1 week acclimation in full strength nutrient solution, rice seedlings were exposed to nutrient solution containing 0, 0.2 or 1.0 mg/L Sb(V) (potassium hexahydroxoantimonate ($KSb(OH)_6$)) for 1 week. They were referred to as Sb0, Sb0.2 and Sb1.0. All experiments were conducted in three replicates. Nine rice seedlings were included in each replicate.

The nutrient solution was complemented with Milli-Q water every 2 d and renewed every 3 d. Ten milliliters of solution were daily taken from each pot for total Sb concentration determination and its speciation analysis. After harvest, plants were collected and carefully washed with Milli-Q water and then divided into roots, stems and leaves. They were liquid nitrogen frozen and refrigerated at −80 °C for further experiments.

2.3. Iron plaque extraction

To analyze Fe and Sb concentrations in iron plaque, dithionite–citrate–bicarbonate (DCB) solution was used to extract iron plaque from the fresh root surfaces (Liu et al., 2004b). Roots were weighed and incubated in 30 mL solution which contained 0.125 M sodium bicarbonate ($NaHCO_3$) and 0.03 M sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) with addition of 0.6 g sodium dithionite ($Na_2S_2O_4$) at room temperature (20–25 °C). After 1 h, roots were taken out and rinsed three times with Milli-Q water. All rinsed Milli-Q water was added to the DCB extracts. The volume of the final solution was fixed to 50 mL with Milli-Q water. The concentrations of Fe and Sb were measured with an atomic absorption spectrophotometer (AAS; PerkinElmer 900T, USA) and inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer NexION 300×, USA), respectively. After DCB extraction, roots were washed with Milli-Q water thoroughly and blotted with tissue papers. They were liquid nitrogen frozen and refrigerated at −80 °C for other analysis.

2.4. Total Sb in plants

The frozen fresh samples were ground in a mortar with liquid nitrogen. Around 0.4 g of plant powder was weighed into the digestion tube, followed by adding 10 mL of 1:1 $HNO_3:H_2O$ to the