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Antimony uptake, translocation and speciation in rice plants exposed to antimonite and antimonate

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HIGHLIGHTS

- We examined the impact of iron plaque on different species of Sb uptake by rice.
- Iron plaque accumulated more Sb than rice plants and affected SbIII and SbV uptake by rice roots.
- Rice was much more efficient in taking up SbIII than SbV, and SbV was the predominant Sb species in rice plants.
- Sb was mostly localized to the cell walls of rice plants, resulting in limited translocation from the roots to shoots.

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ABSTRACT

Antimony (Sb) accumulation in rice is a potential threat to human health, but its uptake mechanisms are unclear. A hydroponic experiment was conducted to investigate uptake, translocation, speciation and subcellular distribution of Sb in rice plants exposed to antimonite (SbIII) and antimonate (SbV) at 0.2, 1.0 or 5.0 mg/L for 4 h. More Sb was accumulated in iron plaque than in the plant, with both the roots (~10–12 times) and Fe plaque (~28–54 times) sequestering more SbIII than SbV. The presence of iron plaque decreased uptake of both SbV and SbIII. SbIII uptake kinetics fitted better to the Michaelis–Menten function than SbV. Antimonate (56 to 98%) was the predominant form in rice plant with little methylated species being detected using HPLC–ICP-MS. Cell walls accumulated more Sb than organelles and cytosol, which were considered as the first barrier against Sb entering into cells. Sb transformation and subcellular distribution can help to understand the metabolic mechanisms of Sb in rice.

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1. Introduction

Antimony (Sb) is considered a priority environmental pollutant by the United States Environmental Protection Agency and the European Union. It has no known biological function and can be toxic at elevated concentrations. Antimonite (SbIII) and antimonate (SbV) are the common species of Sb in the environment and they can be taken up by plants from soil, causing adverse health effects to human. As a metalloid, its environmental behavior has received little attention though it is gaining interest as a global contaminant (Wilson et al., 2010).

The maximum allowable Sb level in drinking water is 5 μg/L in China whereas the World Health Organization (WHO) sets safe drinking water level for Sb at 20 μg/L (He et al., 2012). Anthropogenic activities such as mining, smelting, fossil fuel combustion and waste incineration have elevated Sb levels in the environment. Landrum et al. (2009) recently reported values of 1.12–4.19 mg/L Sb in water from El Tatio Geyser field in Chile. The Sb concentrations in the seepage water from leakage of a smelter in Hunan, China are elevated, ranging from 8.4 to 11 mg/L (He, 2007). The Sb concentration in paddy soils near Xikuangshan Sb mine area in Hunan, China reached 1565 mg/kg (He and Yang, 1999), which was much greater than 36 mg/kg, the maximum permissible pollutant concentrations for Sb recommended by WHO in soils (Chang et al., 2002). Rice plants accumulate high concentration of Sb up to 225 mg/kg in the roots and 5.79 mg/kg in the seeds. Hence, it is important to study Sb behavior in the environment.

Rice is a major food crop for 3 billion people, especially in Asian countries. Rice has been implicated as a major route for Sb exposure, especially in mining areas. The Sb concentrations in rice near the Xikuangshan Sb mine were 160–930 μg/kg (Wu et al., 2011b). According to WHO, rice contributes 33% of the total daily intake of Sb, which is higher than other exposure routes. However, limited data are available regarding Sb uptake and translocation in rice plants. Sb negatively impacts rice growth, with rice yield dropping by 10% when SbIII and SbV concentrations are 150 and 300 mg/kg in soils (He and Yang, 1999).

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Feng et al. (2011a) found that more Sb is concentrated in rice shoots than roots after 14 d exposure to 5 mg/L SbIII under hydroponic conditions. However, SbIII can be oxidized to SbV rapidly in solution. So it is necessary to study SbIII and SbV uptake by rice in short term to minimize SbIII transformation in solution.

Mechanisms of arsenic uptake have been studied extensively in rice plants (Meharg and Jardine, 2003; Zhao et al., 2010). Arsenite (AsIII) and arsenate (AsV) are taken up by aquaporin channels and phosphate transporters by rice, with AsV being reduced to AsIII in root cells. By contrast, little is known about the mechanism of uptake, speciation, and transformation of Sb in rice plants. Okkenhaug et al. (2012) found that SbV is the main species in rice roots and shoots, with >90% of Sb being SbV in porewater in pots. He and Yang (1999) investigated SbIII and SbV accumulation in rice without considering Sb speciation. Huang et al. (2011) studied the influence of Fe plaque on Sb uptake and translocation in rice without considering Sb speciation. It is known that SbIII is more toxic than SbV, so it is necessary to understand Sb transformation in rice to better assess its toxicity.

In addition, subcellular distribution of toxic elements can help to understand their translocation and detoxification mechanism in plants. Cr is mainly associated with cell walls in rice plants (Zeng et al., 2011) so is most of Cd (He et al., 2008). However, information about the subcellular distribution of Sb in rice has rarely been documented.

As a waterlogged plant, rice grows in anaerobic environment and releases oxygen to its rhizosphere through developed aerenchyma (Winkel et al., 2013). The oxygen oxidizes ferrous iron (FeII) to form iron plaque coating on the root surfaces (Zhao et al., 2010). Iron plaque has been shown to have a high affinity for AsV, playing an important role in As uptake by rice. Fe plaque may be responsible for AsII oxidation to AsV, reducing As toxicity (Zhao et al., 2009). Sb and As are chemical analogs so we hypothesized that Sb uptake and speciation in rice was similar to As. Huang et al. (2011) reported that Sb accumulation by rice is influenced by Fe plaque on root surface, with 40–80% of total Sb being accumulated in Fe plaque. However, the direct role of iron plaque in Sb uptake into rice roots needs further investigation.

The overall goal of this study was to examine the uptake, translocation and speciation of Sb by rice plants exposed to SbIII and SbV. Our specific objectives were to: 1) evaluate the effects of iron plaque on Sb accumulation in rice plants; 2) investigate Sb distribution and speciation in rice plants; and 3) study Sb subcellular distribution in rice plants.

2. Materials and methods

2.1. Germination and cultivation of rice plants

Rice seeds (*Oryza sativa* L., Nanjing 45) were surface sterilized by soaking them in 30% H_2O_2 solution for 15 min and then rinsed in Milli-Q water. They were then soaked in Milli-Q water for 48 h, and germinated on moistened filter papers placed in a petri dish. After germination, they were transferred to a 96-orifice plate. At one-leaf stage, they were treated with 0.25-strength nutrition solution recommended by International Rice Research Institute (Wu et al., 2011a). At three-leaf stage, they were treated with 0.5-strength nutrition solution for 1 wk before using full strength nutrition solution.

The nutrient solution pH was adjusted to 5.5–5.8 with KOH. The solution was changed twice a week. The seedlings were acclimated in nutrient solution for 3 wk before treatment. They were grown in a greenhouse with 16 h light period and 8 h dark period using sodium lamps at 180–240 μmol/m^2 s. The temperature was kept at ~28 °C during the day and ~20 °C during the night and relative humidity was maintained at 60–70%.

2.2. Short term uptake of SbIII and SbV by rice plants

After 3-wk acclimation, uniform rice seedlings were washed with deionized water and placed in a 550 mL solution containing 0.5 mM $CaCl_2$ and 0, 0.2, 1, or 5 mg/L SbV (potassium hexahydroxoantimonate ($KSb(OH)_6$)) or SbIII (potassium antimonyl tartrate trihydrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$)) for 4 h (pH 6.0). Aliquots of 10 mL of the solution were taken before and after the experiment for total Sb and Sb speciation measurement. After 4 h, plants were collected and carefully rinsed with Milli-Q water. The plants were divided into roots, stems and leaves. They were flash-frozen in liquid nitrogen and stored at −80 °C for further analysis.

After harvest, all roots were then rinsed with an ice-cold phosphate buffer solution including 1 mM K_2HPO_4, 5 mM MES and 0.5 mM $Ca(NO_3)_2$ for 20 min to remove apoplastic Sb. The colored iron plaques were visible on the root surface. To analyze Fe and Sb concentrations in the iron plaque, fresh root surfaces were extracted using dithionite–citrate–bicarbonate (DCB; Liu et al., 2004). Roots were weighed and incubated for 1 h at room temperature in a 40 mL solution containing 0.125 M $NaHCO_3$ and 0.03 M $Na_3C_6H_5O_7 \cdot 2H_2O$ with addition of 0.8 g $Na_2S_2O_4$. After extraction, roots were rinsed three times with Milli-Q water, which were then added to the DCB extract. The resulting solution was made up to 50 mL with Milli-Q water. The Fe and Sb concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer NexION 300X, USA) after dilution.

2.3. Impact of Fe plaque on Sb uptake kinetics by excised rice roots

Rice roots were excised at the basal node. Half were cleaned with Milli-Q water and incubated in DCB solution for 1 h to remove the iron plaque on the roots. They were then rinsed with Milli-Q water and blotted with tissue paper. All roots were placed in 50 mL plastic tubes containing 5.0 mM 2-(N-morpholin) ethansulfonic acid (MES), 0.5 mM $Ca(NO_3)_2$, and 0–165 mg/L SbIII or 0–251 mg/L SbV (pH 6.0). After 30 min of Sb uptake, all roots were rinsed with an ice-cold phosphate buffer solution and roots with iron plaque were rinsed with 50 mL DCB solution. They were then washed with Milli-Q water and blotted dry. All roots were flash-frozen in liquid nitrogen and stored at −80 °C for further analysis.

2.4. Antimony speciation in rice plants

Frozen plant samples were ground to powder in a mortar under liquid nitrogen for total Sb analysis. ~0.4 g of fine plant powder was weighed into digestion vials, mixed with 10 mL of 1:1 HNO_3:water and left overnight. The digestion was performed using the Hot Block Digestion System (Environmental Express, USA). They were heated at 105 °C for 2 h, removed from the block and cooled for 3 min. The samples were added with 1 mL 30% H_2O_2 slowly, and then heated for another 15 min. The samples were cooled completely, and then diluted up to 50 mL with Milli-Q water. The final solution was stored at 4 °C before analysis with ICP-MS. The blank and certified reference material for rice samples (GSB-21, Chinese geological reference materials) was used for quality control. The mean ± standard error was 4.68 ± 0.36 μg/kg for the sample, which was comparable with the certified value of 4.5 μg/kg. The internal standards were carried out to ensure accuracy and precision. Standard solution at 1 μg/L Sb was measured every 20 samples to monitor the stability of ICP-MS.

The frozen rice samples were crushed in a mortar under liquid nitrogen for Sb speciation (Okkenhaug et al., 2012). ~0.4 g of sample was weighed into a 15 mL polypropylene centrifuge tube and 5 mL of 0.1 M citric acid was added. They were shaken (100 rpm, 25 °C) for 4 h and then sonicated for 1 h. Extracts were centrifuged at 4000 rpm for 15 min, and then collected in a 50 mL centrifuge tube. The residue was rinsed twice with 5 mL of 0.1 M citric acid and all extracts were mixed, and then diluted to 20 mL with Milli-Q water. After filtering using 0.45 μm nylon filter membrane, Sb speciation was measured by high performance liquid chromatography (HPLC; Waters 2695, USA) coupled with ICP-MS. The aqueous solution samples were stored at 4 °C and analyzed within 24 h (Lindemann et al., 2000).