



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



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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.

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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 indispensable 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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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% H₂O₂ 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-orifice plates, which floated on solution containing 0.5 mM Ca(NO₃)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 NH₄NO₃, 10 mg/L of P as NaH₂PO₄, 40 mg/L of K as K₂SO₄, 40 mg/L of Ca as CaCl₂, 40 mg/L of Mg as MgSO₄, 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 μmol/(m²·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 (NaHCO₃) and 0.03 M sodium citrate (Na₃C₆H₅O₇·2H₂O) with addition of 0.6 g sodium dithionite (Na₂S₂O₄) 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 HNO₃:H₂O to the

tube and mixing with the powder. The mixture was allowed to stand overnight for digestion. On the next day, tubes were placed on the Hot Block Digestion System (Environmental Express, USA). The samples were heated at 105 °C for 2 h, and then removed from the block and cooled for 3 min. One milliliter of 30% H₂O₂ was slowly added to the sample, and it was heated for additional 15 min. The digestion solution was completely cooled and added with Milli-Q water up to 50 mL. The final solution was stored at 4 °C before analysis (Srivastava et al., 2010). The Sb concentration was measured with ICP-MS. The blank and certified reference material (GSB-21, also called Liaoning Rice, Chinese Geological Reference Materials) was used for verifying the accuracy and precision of the digestion and the following analyzing procedure. The Sb recovery of the reference material was (104 ± 8) %. The internal standards were used to monitor the instrument signal drift and matrix effect.

2.5. Sb speciation in plants

The fresh sample was pretreated for Sb speciation analysis after Okkenhaug et al. (2012). After ground in a mortar with liquid nitrogen, approximately 0.4 g of plant sample was weighed into a 15 mL polypropylene centrifuge tube with addition of 5 mL 0.1 M citric acid. The mixture was shaken (100 rpm) at 25 °C for 4 h, sonicated for 1 h, and centrifuged (4000 rpm) for 15 min. The supernatant was collected in a 50 mL centrifuge tube. The above steps were repeated twice for the residue. The supernatant from the last two operations was collected in the 50 mL centrifuge tube. Volume of the final solution was fixed to 20 mL with Milli-Q water and passed through a 0.22 μm nylon filter. The filtrate was refrigerated at 4 °C and analyzed within 24 h. The Sb speciation was measured with high performance liquid chromatography (HPLC; Waters 2695, USA) coupled with ICP-MS.

The Sb species analysis was performed using a method developed in a previous study after slight modification (Liu et al., 2010). To separate Sb species, a guard column (Hamilton, UK) connected to a PRP-X100 10 μm anion-exchange column (Hamilton, UK) was used. The mobile phase contained 10 mM EDTA and 2 mM potassium hydrogen phthalate, and its pH was adjusted to 4.5 with ammonium hydroxide. After sonicated and filtered through 0.22 μm nylon membrane, the mobile phase was allowed to flow into the chromatographic column. The sample injection volume was 50 μL. The flow rate was 1.2 mL/min and column temperature was 40 °C. The retention time of Sb(V) and Sb(III) was obtained by running 10 μg/L standard solution, respectively. The 1000 mg/L stock solution of Sb(V) and Sb(III) was prepared from K₂Sb(OH)₆ (Fluka, 99%) and C₈H₄K₂O₁₂Sb₂·H₂O (Sigma–Aldrich, 99%) with Milli-Q water. The stock solution was stored at 4 °C under photo-phobic condition. To establish standard curve, the stock solution was diluted with 0.1 M citric acid to standard solution of different concentrations on the day of analyzing samples. A 100 mg/L multi-element environmental calibration standard (PerkinElmer, in 5% HNO₃) was used to calibrate the standard solution. The blank samples spiked with 10 mg/L Sb(V) or Sb(III) were subjected to the same extraction and analyzing procedure to ensure the accuracy and precision (Zhao et al., 2015). The recoveries of Sb(V)-spiked and Sb(III)-spiked samples were (86 ± 5) % and (81 ± 3) %, respectively. No obvious changes in Sb species were observed after pretreatments.

2.6. Lipid peroxidation

Malondialdehyde (MDA) generated from polyunsaturated fatty acid peroxides was measured to describe lipid peroxidation in roots and leaves of samples. Thiobarbituric acid (TBA) method was used to detect the amount of MDA after Shri et al. (2009). Briefly, around

0.5 g of sample was homogenized with 2 mL 0.5% trichloroacetic acid (TCA). The homogenate was centrifuged at 11,000 g for 15 min. 0.5 mL of the supernatant was taken and mixed with 2.5 mL 20% TCA which contained 0.5% TBA. The mixture was heated in boiling water bath for 30 min and then cooled immediately in ice bath. The absorbance of supernatant at 532 nm was determined with UV–visible spectrophotometer (Shimadzu, Japan). The amount of MDA was calculated with a molar extinction coefficient of $1.56 \times 10^5 / (\text{M} \cdot \text{cm})$.

2.7. Data analysis

Element concentrations in our study were calculated on the basis of fresh weight (Xu et al., 2007). Concentration percentages of Sb in iron plaque and roots, translocation factor (TF) from root to stem and from stem to leaf were calculated as follows:

$$\text{Iron plaque-Sb (\%)} = \frac{C_{\text{DCB-Sb}}}{C_{\text{DCB-Sb}} + C_{\text{Root-Sb}} + C_{\text{Stem-Sb}} + C_{\text{Leaf-Sb}}} \times 100\%$$

$$\text{Root-Sb (\%)} = \frac{C_{\text{Root-Sb}}}{C_{\text{DCB-Sb}} + C_{\text{Root-Sb}} + C_{\text{Stem-Sb}} + C_{\text{Leaf-Sb}}} \times 100\%$$

$$\text{TF}_{\text{root-stem}} = C_{\text{Stem-Sb}} / C_{\text{Root-Sb}}$$

$$\text{TF}_{\text{stem-leaf}} = C_{\text{Leaf-Sb}} / C_{\text{Stem-Sb}}$$

where $C_{\text{DCB-Sb}}$, $C_{\text{Root-Sb}}$, $C_{\text{Stem-Sb}}$, $C_{\text{Leaf-Sb}}$ represent Sb concentrations in DCB extracts, roots, stems and leaves, respectively.

2.8. Statistical analysis

All results are presented as mean ± standard error of three replicates, each with nine plants. Data were analyzed with one-way analysis of variance (ANOVA) and then S–N–K post hoc test. Significant difference was determined at $p < 0.05$ and highly at $p < 0.01$.

3. Results and discussion

3.1. Effects of Sb(V) exposure on growth of rice seedlings

The concentration and speciation of antimony in the nutrient solution were monitored during 7 d exposure. During the exposure period, Sb concentrations were almost unchanged compared to the initial ones. No Sb(III) was detected from the nutrient solution, indicating that no speciation changes of Sb(V) occurred.

All rice seedlings survived under all treatments after 7 d exposure. The shoot part of plant includes stems and leaves. To help understand influence of Sb(V) exposure on growth (biomass and length) of four tested rice cultivars, the harvested plant was divided into shoot and root parts, and the biomass and length of these two parts were measured and compared. The results are presented in Fig. 1. The root biomass of cultivar N respectively decreased by (42 ± 7.5) % and (31 ± 7.9) % at low [Sb(V)0.2] and high [Sb(V)1.0] exposure levels to Sb(V), and its shoot biomass correspondingly decreased by (27 ± 5.8) % and (33 ± 16.4) % as compared to the blank control. Sb(V) exposure respectively reduced the root biomass of cultivar F by (35 ± 9.1) % and (23 ± 9.4) % at low and high levels in contrast to the control, while no significant effects on shoot biomass were observed. Relative to the blank control, root and shoot biomasses of the cultivar G decreased by (26 ± 2.4) % and (13 ± 6.8) % at low exposure level to Sb(V), respectively. Correspondingly, they only reduced by (13 ± 6.2) % and (5.9 ± 1.5) % at high exposure level. The above mentioned findings suggested that

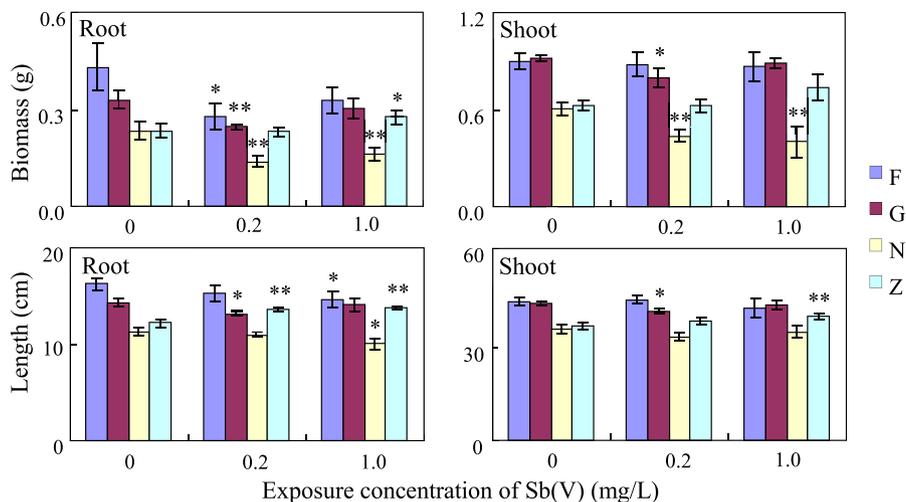


Fig. 1. Effect of Sb(V) exposure on growth (biomass and length) of four cultivars at levels of 0, 0.2 and 1.0 mg/L. Significant differences between treatment groups and the control are indicated as * ($p < 0.05$) and ** ($p < 0.01$).

biomass of rice seedlings of the cultivars N, F and G was differently and negatively influenced after exposed to Sb(V), depending on their species difference. The biomass reduction resulting from exposure to Sb(V) was not closely related to the exposed concentrations, and the reduction at lower exposure concentration (0.2 mg/L) was higher than that at higher one (1.0 mg/L) in some cases for a given rice cultivar. This could imply that the exposed Sb(V) did not exhibit strong toxic effect on growth of rice cultivars even through its concentration was increased to 1 mg/L. It was evident that growth (biomass) of the japonica rice (cultivar N) was more strongly inhibited resulting from exposure to Sb(V) than the indica hybrid rice (cultivars F and G) (Fig. 1). The reduction in root biomass associated with an increase in root MDA content indicated that Sb was toxic to rice seedlings (Figs. 1 and 2). For cultivar Z, its root and shoot biomasses were almost unaffected at low exposure level. However, it was interesting to note that, different from the cultivars N, F and G, root and shoot biomasses of the cultivar Z respectively increased by $(18 \pm 9.4)\%$ and $(25 \pm 1.4)\%$ as compared to the blank control at high level of Sb(V), which could be due to the hormesis effect (Ren et al., 2014b). For all cultivars, the difference in root and shoot lengths between treatment groups and the blank control was in accordance with that of root and shoot biomasses (Fig. 1). This suggested that Sb(V) exposure had quite similar influence on both length and biomass of rice seedlings.

3.2. Antimonate exposure caused lipid peroxidation

To better understand toxicity of Sb(V) to rice, root and leaf samples were analyzed for determination of MDA content. The

formation of reactive oxygen species (ROS) in plants can be stimulated by metal (loid)s, such as As, Cd and other metals (Gratao et al., 2005; Singh et al., 2007). Once the oxidative stress is too heavy for antioxidant defense system to bear, lipid peroxidation would occur, which is a physiological reaction to the oxidative damage. As a lipid peroxidation product, MDA was generated from peroxidation of unsaturated fatty acids in the membranes (Shri et al., 2009).

The MDA content in the roots of japonica rice (cultivars N and Z) significantly increased with increasing exposure concentration of Sb(V), whereas that of indica hybrid rice (cultivars F and G) did not change so much (Fig. 2). Particularly, after exposed to the low and high levels of Sb(V), the MDA contents in cultivar N root increased by $(125 \pm 31)\%$ and $(155 \pm 1)\%$, and they respectively increased by $(30 \pm 18)\%$ and $(40 \pm 6)\%$ in the root of cultivar Z as compared to their control samples. It was evident that for the two japonica rice N and Z, exposure to Sb(V) at a low level (0.2 mg/L) would considerably elevate their MDA content in root. The difference in MDA content in the roots of four rice cultivars indicated that accumulation of ROS and lipid peroxidation of the japonica rices N and Z induced by exposure to Sb(V) were more pronounced relative to the indica hybrid rice F and G, thus posing a greater threat to their development and growth. This could be the reason why the biomass of the cultivars F and G was less effected by exposure to Sb(V) as stated above. It was observed in a previous study that about 60 mg/kg of Sb accumulated in rice leaves caused 9.9% enhancement of MDA production and 14% decrease of biomass (Feng et al., 2011). The Sb accumulation in rice leaves as reported by Feng et al. (2011) was much higher than that observed in the

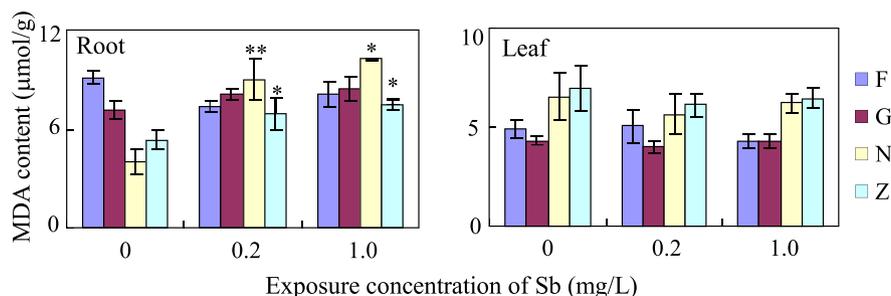


Fig. 2. MDA content in roots and leaves of four cultivars after exposed to 0, 0.2 or 1.0 mg/L Sb(V). Significant differences between treatment groups and the control are indicated as * ($p < 0.05$) and ** ($p < 0.01$).

present work as four tested rice cultivars were exposed to Sb(V) at low and high levels with values respectively being 0.04–0.60 mg/kg and 0.36–1.86 mg/kg. However, the biomass reduction observed by the authors was lower than that of ours. This could be a result that the rice species they used was Weiyou 402, a kind of indica hybrid rice, which was different from those used in the present study. They could have dissimilar tolerance to Sb(V) exposure.

Differently, no significant difference in MDA content in leaves of all four cultivars with and without Sb(V) exposure was observed (Fig. 2), noting that the negative effect of Sb(V) exposure on development and growth of the tested rice roots was more pronounced than the leaves. This was mainly because rice roots were the first contact point of Sb(V) in the nutrient solution and much higher Sb was accumulated in rice roots than in leaves (Fig. 3). Consistent with our observation, a study by Corrales et al. (2014) showed that 200 μ M Sb supply significantly increased the root MDA content while had no effects on shoot MDA content.

3.3. Effects of cultivars on iron plaque formation

After 7 d exposure, the reddish iron plaque was clearly visible at the root surface. As an index of the amount of iron plaque, the mass concentration of iron plaque formed at the root surfaces of cultivar N was as high as 2–4 times of others (Fig. 4). Correspondingly, the color of iron plaque at the root surfaces of cultivar N was much darker relative to others (Fig. 5).

Two underlying conditions are required for iron plaque formation at the root surfaces of plants: (1) the local oxidizing environment; and (2) the plentiful iron element supply in the growth medium (Chen et al., 1980). As Fe(II) concentration in the nutrient solution was identical under all treatments, the amount of iron plaque created at the root surfaces was determined by the amounts of oxygen and oxidants released through the developed aerenchyma. As the amounts of oxygen and oxidants are very limited, only the Fe(II) at the root surfaces or in the rhizosphere can be oxidized to Fe(III). However, under the condition with enough oxygen and oxidants, the Fe(II) in nutrient solution can be oxidized to Fe(III) as well, rendering additional Fe(III) precipitate on root surfaces. This can well explain why the nutrient solution of cultivar N was a little yellow brown during the exposure period. The relatively darker color of iron plaque at the root surfaces of cultivar N relative to others implied that it is a rice species with higher oxidizing capability for Fe(II) among all tested cultivars, particularly with higher transmission capability of oxygen to root and stronger secretion ability of the oxidizing substances from root.

At low exposure level of Sb(V) (Sb0.2), the mass concentration of iron plaque formed at the roots of cultivars F, G, N, Z were $(64 \pm 5)\%$, $(44 \pm 9)\%$, $(75 \pm 8)\%$ and $(34 \pm 15)\%$ higher than the corresponding control samples, suggesting that Sb(V) exposure greatly enhanced

iron plaque formation for all tested rice cultivars. In comparison, at high exposure level of Sb(V) (Sb1.0), the mass concentration of iron plaque at the root surfaces of cultivars F and N was almost doubled as compared to the control (Fig. 4). The elevation in mass concentration of iron plaque could stem from the increase in the total amount of iron plaque or the root biomass reduction. The total amount of iron plaque at the root surfaces of cultivars N and Z tended to be significantly enhanced with increasing exposure concentration of Sb(V) (Fig. 6). This can be supported by the fact that MDA content in the roots of both cultivars N and Z increased with increasing exposure concentration of Sb(V). MDA as a biomarker of lipid peroxidation originates from the peroxidation of unsaturated fatty acids, which is induced by ROS (Shri et al., 2009). An increase in MDA content in the roots of both cultivars N and Z induced by the elevation of the exposure concentration of Sb(V) implied that a larger quantity of ROS and oxidizing substances were generated in their roots (Singh et al., 2007), which in turn enhanced iron plaque formation. Mirroring the fact that MDA contents in the roots of both cultivars F and G at low and high exposure levels of Sb(V) were comparable (Fig. 2), the total amount of iron plaque at their root surfaces slightly increased as the exposure concentration of Sb(V) was increased (Fig. 6).

3.4. Influence of iron plaque on uptake of antimony

The accumulated concentration of Sb(V) in iron plaque at the root surfaces significantly increased with increasing mass concentration of iron plaque under both low and high Sb(V) exposure conditions (Fig. 7), noting that a larger amount of Sb(V) was intercepted in iron plaque at the root surfaces of rice. Similar phenomenon was observed for As in a previous study (Liu et al., 2004a,b). It was further documented in a review paper that, as the pH of solution was 6.0, Sb(V) mainly existed as $[\text{Sb}(\text{OH})_6]^-$, which can be sorbed at the protonated surface sites of the iron plaque (Wilson et al., 2010). As cultivar N was the highest in iron plaque formation among four cultivars, the Sb concentration accumulated at its surfaces under low Sb(V) exposure condition was as high as 2–3 times of other three cultivars (Fig. 4). In comparison, accumulation of Sb(V) at the root surfaces of the rest three rice cultivars was not significantly different. As the exposure concentration of Sb(V) was increased to 1.0 mg/L, Sb concentration at the root surfaces of all four cultivars significantly increased (Fig. 4), suggesting the increasing enhancement effect on Sb accumulation at root surfaces.

Our experimental observation showed that the concentration percentage of Sb in rice seedling roots significantly decreased with an increase in that accumulated in the iron plaques (Fig. 7), indicating that the iron plaques may act as a “buffer” to Sb uptake. A previous study also consistently demonstrated that the presence of

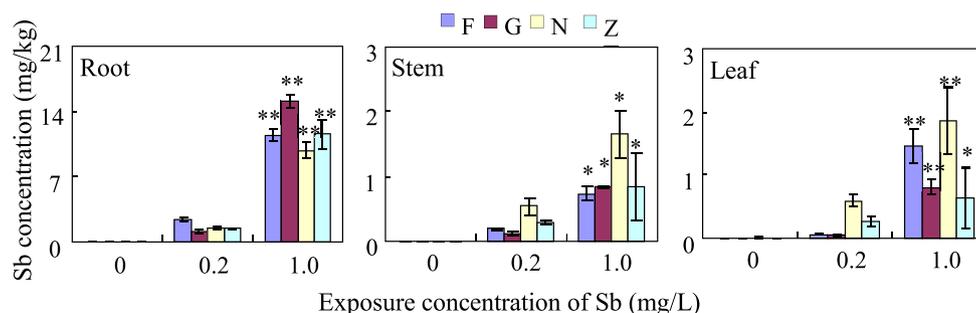


Fig. 3. Sb concentration in roots, stems and leaves of four cultivars after exposed to 0, 0.2 or 1.0 mg/L Sb(V). Significant differences between treatment groups and the control are indicated as * ($p < 0.05$) and ** ($p < 0.01$).

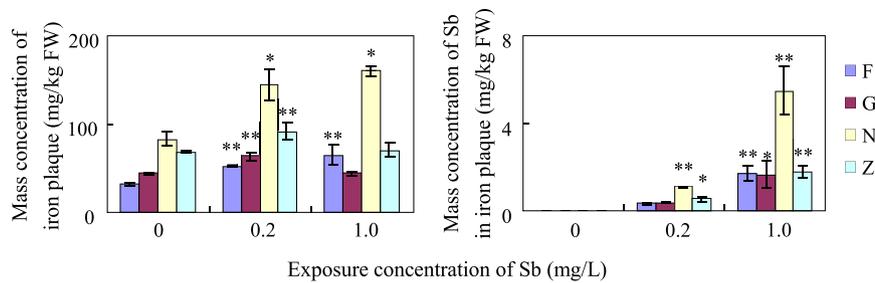


Fig. 4. Mass concentration of iron plaque (mg/kg FW) at root surfaces and that of Sb (mg/kg FW) in iron plaque of four cultivars after exposed to 0, 0.2 or 1.0 mg/L Sb(V). Significant differences between treatment groups and the control are indicated as * ($p < 0.05$) and ** ($p < 0.01$).

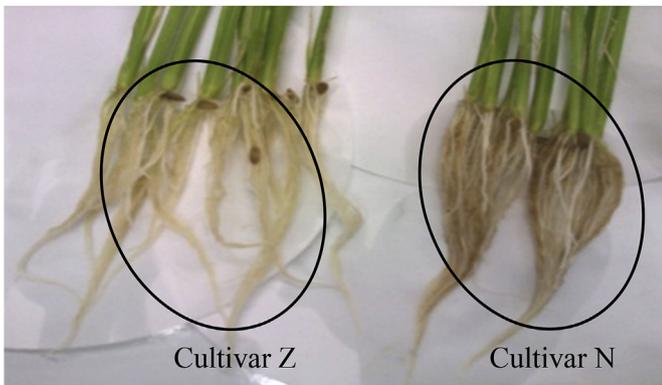


Fig. 5. Images showing the iron plaque formed at the root surfaces of the rice cultivars N and Z exposed to Sb(V) at a low concentration of 0.2 mg/L. The color of the root surface of the cultivars F and G was comparable and very similar to that of cultivar Z. The photos for these two cultivars were not taken. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

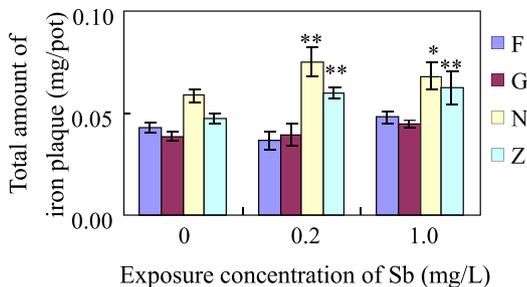


Fig. 6. Total amount of iron plaque (mg/pot) of four cultivars after exposing to 0, 0.2 or 1.0 mg/L Sb(V). Significant differences between treatment groups and the control are indicated as * ($p < 0.05$) and ** ($p < 0.01$).

iron plaque decreased uptake of arsenate by rice seedling roots (Chen et al., 2005). In a hydroponic experiment, Cui et al. (2015) observed that the presence of iron plaque significantly reduced uptake of Sb(V) by rice roots as well. Ren et al. (2014a) examined Sb(V) uptake kinetics using excised rice roots, and similar phenomenon was observed. It has been demonstrated that iron hydroxides had high sorption affinity for Sb(V) (Leuz et al., 2006), so it may be largely “locked up” in iron plaque.

3.5. Antimony distribution in different plant tissues

For all four cultivars, the accumulated total concentrations of Sb in roots were the highest under both low and high Sb(V) exposure concentrations among roots, stems and leaves. In comparison, the

total concentrations of Sb in both stems and leaves were comparable (Fig. 3). As the exposure concentration of Sb was increased from 0.2 mg/L to 1.0 mg/L, the total concentrations of Sb in roots, stems and leaves steeply rose, and those in roots were the most pronounced (Fig. 3). It was shown in a previous study that As(V) was able to penetrate cell membrane through the phosphate channel (Asher and Reay, 1979). However, Sb(V) cannot share the phosphate pathway, because both arsenate and phosphate have the tetrahedral structure, while the structure of Sb(V) is octahedral which is larger and has lower charge density (Meharg and Hartley-Whitaker, 2002). The Sb(V) may enter into the plant root via anion transporters or through the apoplastic pathway by passing the not-well-developed or damaged casparian strip (Tschan et al., 2008).

Translocation factor (TF) was used to better understand the distribution of Sb in rice seedlings. It is a valid parameter to estimate whether the cultivar is suitable to be planted on sites contaminated by Sb. TFs of Sb from root to stem of all four cultivars at low exposure level ranged from 0.1 to 0.3, indicating that a fraction of Sb taken up by root was translocated to stem (Fig. 8). Furthermore, Sb translocation from root to stem of japonica rice (cultivars N and Z) was more evident than the indica hybrid rice (cultivars F and G). TFs from root to stem generally decreased with increasing exposure concentration of Sb(V), with an exception that the TF of cultivar F increased a little bit. Translocation of Sb from stem to leaf of Cultivar N was the strongest at low exposure level of Sb(V) (Fig. 8). The TF from stem to leaf of cultivars F and Z at high exposure level were 4 and 2 times higher than that at low level, respectively. In comparison, the TF of cultivars G and N was rather stable as the exposure level of Sb(V) was increased.

Sb accumulation in rice shoots may threaten human health through the products of livestock fed on rice straws. Furthermore, a cultivar with relatively higher concentration of Sb in shoots would most likely have higher Sb concentration in grains, which may pose higher health risks to the populations upon consumption of rice. Although cultivar N had the lowest root Sb concentration among all studied cultivars, it was not suitable for planting on soils contaminated by Sb due to its strongest translocation tendency of Sb from root to stem. Due to very high TF value of Sb from stems to leaves of cultivar F especially at high exposure level (Fig. 8), its leaf concentration was 22% lower than that of cultivar N, but was 2 and 4 times of the cultivars G and Z (Fig. 3). Hence, the key point one needs to be aware that, it is inappropriate to grow cultivar F in soils with available Sb concentration over 1.0 mg/kg, since translocation of Sb from stem to leaf was sharply enhanced with an increase in its exposure concentration.

3.6. Antimony speciation in rice plants

Antimonate remained stable in nutrient solution during the 7 d exposure. Sb(III) was detected from the roots, stems and leaves of

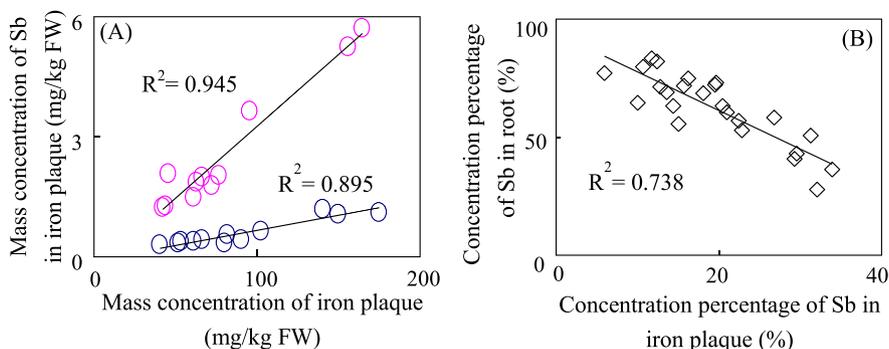


Fig. 7. (A) The relationship between Sb and Fe concentrations in DCB extracts. Blue symbols represent the Sb exposure concentration at 0.2 mg/L ($R^2 = 0.895$). Red ones refer to the Sb exposure concentration at 1.0 mg/L ($R^2 = 0.945$). (B) The relationship between the concentration percentages of Sb in roots and in iron plaque ($R^2 = 0.738$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

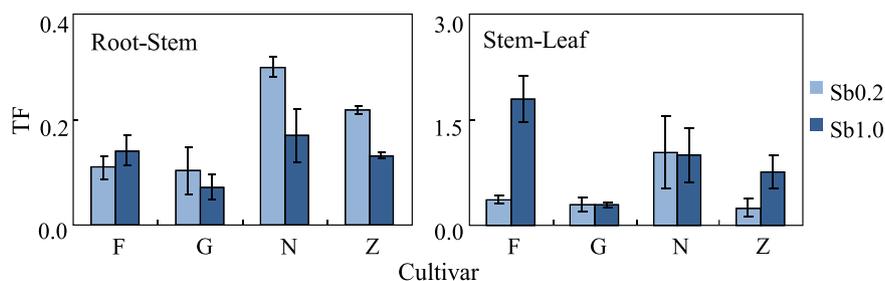


Fig. 8. The translocation of Sb in different tissues of rice seedlings for four cultivars.

all rice cultivars after exposure. About 6–13%, 10–26% and 4–15% of total Sb was present as Sb(III) in roots, stems and leaves, respectively, while exposed to 0.2 mg/L of Sb(V) (Table 1). Sb(III) was also detected from these parts at 1.0 mg/L of Sb(V) exposure level, but with lower percentages being about 2–6%, 10–13% and 3–10%. For all cultivars, Sb(III) percentage in stems was the highest, which was consistent with a previous report (Okkenhaug et al., 2011). Since the tested rice was only exposed to Sb(V), the Sb(III) detected from various parts of plant stemmed from the reduction of Sb(V) after it was taken up. It has been shown that As(V) was reduced to As(III) in cells by arsenate reductases (Zangi and Filella, 2012). Furthermore, As(V) can compete with P in cells, which in turn may interfere the tricarboxylic acid cycle (Meharg, 1994). Due to the fact that Sb is an analog of As, the metabolism mechanism of Sb(V) in rice could be similar to that of As(V). Up to now, LmACR2 from *Leishmania major* is the only known antimonate reductase for Sb(V) (Zhou et al., 2004). However, its specific metabolism mechanism still remains unclear, which needs to be explored in the future work.

Interestingly, the Sb(III) percentage decreased with increasing exposure concentration of Sb(V). Similar phenomenon was observed in a recent study showing that As(III) abundance in *Pteris*

vittata root decreased when the exposure concentration of As(V) in nutrient solution was over 300 μ M (Hatayama et al., 2011). Since Sb(III) is more toxic to human than Sb(V), reduction of Sb(V) to Sb(III) in rice tissues would exert extra harmful effect on human health through the wide use of rice straws as forage.

4. Conclusions

Sb(V) exposure reduced the biomass of cultivars F, G and N. Japonica rice (cultivars F and G) was more sensitive to Sb(V) exposure than indica hybrid rice (cultivar N). The reduction in biomass associated with an increase in MDA content indicated toxicity of Sb(V) to rice seedlings. The iron plaque formation at the rice root surfaces varied with cultivars and the amount of iron plaque on the cultivar N root surface was the highest. The total amount of iron plaque formed at the root surfaces of cultivars N and Z significantly increased with increasing Sb(V) exposure concentration. This can be attributed to the fact that a larger quantity of ROS and oxidizing substances were released from their roots, as supported by the consistent increase in MDA content in roots. The iron plaque was able to accumulate Sb and acted as a buffer to Sb

Table 1

The percentage of Sb(III) in total Sb in roots, stems and leaves of rice seedlings.

Sb exposure (mg/L)	Cultivar	Sb(III)% in roots	Sb(III)% in stems	Sb(III)% in leaves
0.2	F	6.14 \pm 0.13	15.60 \pm 1.96	12.37 \pm 0.61
	G	9.06 \pm 0.35	25.92 \pm 0.61	14.90 \pm 3.43
	N	13.25 \pm 0.57	11.99 \pm 2.82	4.48 \pm 1.21
	Z	8.49 \pm 1.67	10.43 \pm 1.24	9.02 \pm 0.74
1.0	F	2.69 \pm 0.53	10.41 \pm 2.17	2.52 \pm 0.47
	G	2.05 \pm 0.19	12.74 \pm 2.29	4.06 \pm 1.33
	N	6.38 \pm 0.11	10.92 \pm 5.22	3.52 \pm 0.16
	Z	2.91 \pm 0.40	10.04 \pm 1.04	9.59 \pm 5.21

uptake. Consequently, cultivar N accumulated the lowest Sb concentration in root among all cultivars. However, the Sb concentration accumulated in the shoots of cultivar N was the highest, due to its strongest translocation. Furthermore, translocation of Sb from stem to leaf of cultivar F was sharply enhanced as the exposure level of Sb(V) was increased. Sb (V) in rice seedlings can be reduced to Sb(III), especially in stems. The accumulation and reduction of Sb(V) in rice seedlings may threaten the food safety and pose significant health risk to human beings through food chain, because rice straws are widely used as forage. Hence, when choosing the suitable cultivars planted on Sb-contaminated soil, both its accumulation and transformation in rice should be taken into consideration.

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