Influence of iron plaque and cultivars on antimony uptake by and translocation in rice (*Oryza sativa* L.) seedlings exposed to Sb(III) or Sb(V)

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Abstract

Background and Aims Characteristically baseline levels of Sb in the environment are low, but problematic local elevation trends arise from anthropogenic activities such as mining and incineration. Arsenic (analog of Sb) accumulation by rice can be reduced by iron (Fe) plaque. A hydroponic experiment was conducted to investigate whether Fe plaque could reduce the uptake and translocation of different Sb species in different rice cultivars.

Methods After Fe plaque on rice roots was induced in solution containing 0, 0.2, 0.4, 0.7, 1.2, 2.0 mM Fe²⁺ for 24 h, seedlings were transferred into nutrient solution with 20 μM Sb(V) or Sb(III) for 3 d.

Results About 60–80% (Sb(III) treatment) and 40–60% (Sb(V) treatment) of the total Sb accumulated in Fe plaque. There was a significant correlation between the concentrations of Sb and Fe on the root

surface. A similar relationship was observed in roots and shoots. Cultivar (Jiahua 1) formed the most Fe plaque, had the highest Fe associated Sb sequestration but the lowest Sb concentration in the root interior.

Conclusions Fe plaque may act as a ‘buffer’ for Sb (V) and Sb(III) in the rhizosphere, and cultivars played an important role in the different species Sb uptake and translocation.

Keywords Antimony species · Translocation · Iron plaque · Rice Cultivars

Introduction

Ranking 62nd in crustal abundance the trace element Antimony is commonly found in soils at concentrations between 0.2 and 0.3 mg kg⁻¹ (Rish 2004). It has no biological function and is non-essential to plants (He and Yang 1999; Miravet et al. 2005). However, antimony is a potent mammalian toxin (Gebel 1997), and considered as a priority pollutant by USA (USEPA 1999) and EU (Council of the European Communities 1976). It has been reported that high concentrations of antimony persist at some sites of the world due to human activities such as mining and incineration (Baroni et al. 2000; Hammel et al. 2000; Flynn et al. 2003). In the latest decades, global fluxes of Sb have increased at least 10-fold (Irina et al. 2011). Plants growing in these Sb-contaminated areas can accumulate antimony from

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the soil (Murciego et al. 2007). China is rich in antimony mine resources. Most antimony mines are located in Hunan and Guangxi Provinces (He and Yang 1999). Rice is the staple food for the people in these areas. So, more attentions should be paid to the uptake by and translocation of antimony in rice from these regions. Furthermore, the toxicity of Sb in the environment strongly depends on the speciation. The general order of the toxicity for Sb species is given as: organoantimonials (e.g. methylated species) < antimonates (Sb(V)) < antimonites (Sb(III)) (Gebel 1997; He and Yang 1999; Krachler et al. 2001; Filella et al. 2002), which is similar to arsenic (As) (Susan et al. 2010). Since inorganic Sb species are more abundant than organic species in the natural systems (Filella et al. 2009), Sb(V) and Sb(III) were used in our experiments.

As a waterlogged plant, rice is usually cultivated in an anaerobic and chemically reduced environment (Colmer 2003). The concentrations of metal(loid) ions in soil, such as Fe^{2+} , Mn^{2+} , Cu^{2+} , Ni^{2+} , arsenite can significantly increase in anaerobic conditions (Armstrong 1967). Rice development can be seriously affected if these reduced metal ions are taken up in excess by roots. Radial oxygen loss (ROL) from roots to the rhizosphere is considered to be essential for detoxification of these phytotoxins by direct oxidation or oxidizing aerobic microorganisms maintained in the rhizosphere region (Armstrong and Armstrong 2005).

Most aquatic plant species, including rice, can form iron plaque on their roots by oxidizing Fe(II) to Fe(III). Some reports have shown that iron plaque can act as a barrier to the uptake of heavy metals, such as Cu, Cd, Mn and Ni (Greipsson 1995; Wang and Peverly 1999; Batty et al. 2000). However, a number of reports conclude the opposite, with Fe plaque found to increase the uptake of toxic and nutrient elements (Zhang et al. 1998; Ye et al. 2001). Therefore the overall effect of Fe plaque on the uptake of toxic and nutrient elements may be related to the amounts of Fe plaque (Zhang et al. 1998).

Arsenic accumulation by rice can be affected by iron plaque (Liu et al. 2004a, b; Chen et al. 2005). Because Sb and As are metalloids belonging to Group 15 of the periodic table of elements, they share some toxicological and chemical properties with As (Casiot et al. 2007; Susan et al. 2010), antimony accumulation by rice may be also influenced by the amounts of Fe plaque formed on the root surface.

Consequently the aims of our study were 1) to investigate whether Fe plaque could affect the different forms of antimony accumulation in different rice cultivars; 2) to reveal the relationship between Fe and Sb in Fe plaque and rice tissues; 3) to investigate the translocation of Sb(III) and Sb(V) in different rice cultivars.

Materials and methods

Plant cultivation

Seeds of three rice (*Oryza sativa* L.) cultivars (Yangdao 6(YD), Nongken 57(NK), Jiahua 1(JH)) were used in our experiment. Seeds were sterilized in 30% H_2O_2 solution for 15 min, followed by thoroughly washing with distilled water. After germination in moist perlite for 3 weeks, uniform seedling were selected and transported to PVC pots (7.5 cm diameter and 14 cm height, one plant per pot). Each pot contained 500 ml 1/3 strength nutrient solution which is modified from Hewitt (1966). The composition of the full strength nutrient solution contained 50 μM Fe(II)-ethylenediaminetetraacetic acid (EDTA), 10 μM H_3BO_4 , 1.0 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 5.0 μM $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.5 μM $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.2 μM $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, 5 mM NH_4NO_3 , 2 mM K_2SO_4 , 4 mM CaCl_2 , 1.5 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.3 mM KH_2PO_4 . The solution pH value was adjusted to 5.5 using 0.1 M KOH or HCl. The nutrient solution was changed every 3 to 4 days.

All pots were placed in a controlled environment growth chamber with a 14 h light period (260–350 $\text{mm}^2 \text{s}^{-1}$) and a 10 h dark period. The temperature was kept at 25°C during the day and 20°C during the night. The relative humidity was 70%.

Experimental treatments

After the rice seedlings grew in nutrient solution for 3 weeks, iron plaque was induced on the roots surface. Before the iron plaque induction, all plants were washed and put into deionized water for 12 h to minimize any disturbance from other elements to iron. They were transferred into 500 ml solution with 0, 0.2, 0.4, 0.7, 1.2, and 2.0 mM of ferrous ion for 24 h (Fe^{2+} as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, pH 5.5). These treatments were called Fe0, Fe0.2, Fe0.4, Fe0.7, Fe1.2, and Fe2.0, respectively.

After iron plaque induction, seedlings were transferred into 1/3 strength normal nutrient solution for 48 h. Then they were transferred in nutrient solution with 20 μM Sb(III) (Potassium antimony(III) oxide tartrate hemihydrate; $\text{KSbC}_4\text{H}_4\text{O}_7 \cdot 1/2\text{H}_2\text{O}$) or Sb(V) (Potassium hexahydroxoantimonate(V); KSb(OH)_6) for 3 d. The solutions were renewed every day to avoid changes in Sb species. Each treatment had three replicates, and there were 108 pots in all.

DCB extraction of iron plaque

At harvest, all roots of seedlings were washed three times with deionized water. Then iron plaque on root surfaces were extracted using dithionite-citrate-bicarbonate (DCB), as described by Liu et al. (2004a). Roots of each seedling were cut and incubated for 1 h at room temperature (20–25°C) in 30 ml solution containing 0.125 M sodium bicarbonate (NaHCO_3) and 0.03 M sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) with the addition of 0.5 g sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$). Roots were rinsed three times with deionized water which was added to the DCB extracts. The final solution was made up to 50 ml with deionized water. After DCB extraction, roots and shoots were oven-dried at 70°C for 72 h and weighed.

Plant digestion and analysis

For determination of total antimony in the roots and shoots, homogenized samples (0.1–0.2 g) were weighed into a 50 ml volume centrifuge tube and soaked in 2 ml of high purity HNO_3 . After standing overnight at room temperature (20–25°C), the samples were randomized and subjected to microwave digestion (Mars, Matthews Inc., USA). The digestion program was run as follows: 55°C for 10 min, 75°C for 10 min, 95°C for 30 min with 5 min ramp time between each stage. The digested samples were diluted to 40 ml with deionized water and then filtered to remove impurities. This method was reported by Sun et al. (2008) for determining total As concentrations.

A reagent blank and standard reference plant material (Bush branches and leaves, GBW07603 from the National Research Center for Standards in China) were included, to verify the accuracy and precision of the digestion procedure and subsequent analysis recovery. The concentrations of Sb in the DCB-

extracts and Fe were measured by ICP-OES. The concentrations of Sb in the acid digests were measured by ICP-MS. The recovery rates of Fe and Sb were $85 \pm 5\%$ and $162 \pm 8\%$ (means $\pm$ SD, $n=6$) in this experiment, respectively.

Statistical analysis

Analyses of variance (ANOVA) were performed using SPSS 16.0.

Results

Iron plaque formation

Iron plaque was clearly visible on the root surface after 24 h Fe^{2+} incubation. The amounts of iron plaque differed between cultivars and increased with increasing of Fe^{2+} concentrations in the pretreatment solutions, while levels kept stable when the increment of iron concentration reached a cultivar specific threshold (Fig. 1). Cultivar YD got the lowest DCB-Fe concentration and maintained a steady Fe level in treatments above Fe0.4. For cultivars NK and JH, the amounts of Fe in plaque increased between Fe0 - Fe0.7, and then kept stable. Moreover, the amounts of Fe in plaque for three cultivars followed the trend of $\text{JH} > \text{NK} > \text{YD}$ ($P < 0.05$) at each iron level.

Fig. 1 The influence of Fe concentrations in pretreatment solution on the amounts of iron plaque formed on the root surface of three cultivars: Yangdao 6 (YD), Nongken 57 (NK) and Jiahua 1 (JH). (means $\pm$ SE, $n=6$)

Sb accumulation in iron plaques

Antimony concentrations in DCB extracts increased significantly with Fe concentration for the three cultivars when Sb(III) (Fig. 2) and Sb(V) (Fig. 3) were supplied. Cultivars, Sb species and Fe^{2+} concentrations in the pretreatment solutions had significant effects on Sb concentrations in iron plaques (Table 1). Sb accumulated in the iron plaques when Sb(III) supplies were much higher than those of Sb(V) for three cultivars (Table 1). Cultivars had a strong influence on Sb concentrations accumulated in iron plaque when Sb(III) was supplied but there was no significant effect with Sb(V).

Effect of iron plaque on uptake and translocation of antimony

Fe^{2+} concentrations in pretreatment solutions had no significant effect on Sb concentrations in roots but had influence in shoots with Sb(III) supplied for three cultivars (Table 2). When the iron concentrations increased, fluctuation of Sb concentrations in shoots was observed. Sb concentrations in roots increased significantly with the increment of Fe^{2+} concentrations in pretreatment solution for cultivars YD and NK in Sb(V) treatment. But no change was found for cultivar JH. Iron concentrations in pretreatment solution had no effect on the Sb concentrations in shoots with Sb(V) addition (Table 2). Sb accumulation in roots and shoots when Sb(III) supplied was much higher than that when Sb(V) supplied for three cultivars (Table 2).

Fig. 2 Correlation between concentrations of Fe and Sb in DCB extracts ($P < 0.01$). Plants were exposed to nutrient solution with Sb(III) for 72 h

Fig. 3 Correlation between concentrations of Fe and total Sb in DCB extracts ($P < 0.01$) (a: cultivar YD; b: cultivar NK; c: cultivar JH). Plants were treated by Sb(V) for 72 h

Cultivars had no effect on the Sb concentrations in shoots either with Sb(V) supplied or with Sb(III). However, the opposite conclusion was made in roots. Cultivar JH make the lowest Sb concentration in root

Table 1 Sb concentrations in DCB extracts of three rice cultivars treated by different Sb species and the analysis of variance (means $\pm$ SE, $n=3$)

Fe Pretreatment	DCB-Sb (mg kg ⁻¹ RDW)		Sb(III) treatment	DCB-Sb (mg kg ⁻¹ RDW)		Sb(V) treatment
	YD	NK		YD	NK	
Fe0	308 $\pm$ 26.1c	578 $\pm$ 15.1 d	784 $\pm$ 43.3 d	71.2 $\pm$ 5.16 d	99.6 $\pm$ 2.05 d	105 $\pm$ 8.43 d
Fe0.2	435 $\pm$ 19.1c	872 $\pm$ 5.28 cd	1291 $\pm$ 66.8c	139 $\pm$ 11.4c	161 $\pm$ 13.6c	147 $\pm$ 3.64c
Fe0.4	760 $\pm$ 87.9ab	1292 $\pm$ 110bc	1839 $\pm$ 96.6b	177 $\pm$ 17.5c	210 $\pm$ 14.1c	185 $\pm$ 5.44b
Fe0.7	675 $\pm$ 52.1ab	1589 $\pm$ 150ab	2279 $\pm$ 52.4a	234 $\pm$ 14.7b	302 $\pm$ 30.3b	235 $\pm$ 7.13a
Fe1.2	838 $\pm$ 126a	1832 $\pm$ 295a	2532 $\pm$ 106a	284 $\pm$ 2.85a	342 $\pm$ 25.4ab	189 $\pm$ 4.44b
Fe2.0	595 $\pm$ 66.2b	1759 $\pm$ 120a	2396 $\pm$ 126a	313 $\pm$ 20.1a	374 $\pm$ 18.4a	253 $\pm$ 12.2a
Analysis of Variance						
Cultivars (C)		$P<0.01$			NS	
Fe		$P<0.01$			$P<0.01$	
Sb Speciation (Sb)		$P<0.01$			$P<0.01$	
C $\times$ Fe		$P<0.01$			$P<0.01$	
C $\times$ Sb		$P<0.01$			$P<0.01$	
Fe $\times$ Sb		$P<0.01$			$P<0.01$	
C $\times$ Fe $\times$ Sb		$P<0.01$			$P<0.01$	

Table 2 Sb concentrations in roots and shoots of three rice cultivars treated by different Sb species and the analysis of variance (means $\pm$ SE, $n=3$)

Sb Treatment	Fe Pretreatment	Sb in roots (mg kg ⁻¹ RDW)			Sb in shoots (mg kg ⁻¹ SDW)		
		YD	NK	JH	YD	NK	JH
Sb(III)	Fe0	270 $\pm$ 13.3a	332 $\pm$ 26.3a	204 $\pm$ 10.9a	15.4 $\pm$ 2.87ab	16.3 $\pm$ 2.56a	10.7 $\pm$ 2.29 ac
	Fe0.2	301 $\pm$ 28.9a	307 $\pm$ 27.8a	203 $\pm$ 2.78a	12.9 $\pm$ 1.19ab	11.7 $\pm$ 1.38ab	6.77 $\pm$ 0.30c
	Fe0.4	292 $\pm$ 10.3a	300 $\pm$ 10.1a	220 $\pm$ 1.54a	17.1 $\pm$ 1.99a	7.07 $\pm$ 0.84b	8.04 $\pm$ 0.84bc
	Fe0.7	357 $\pm$ 11.8a	299 $\pm$ 26.7a	194 $\pm$ 22.7a	14.5 $\pm$ 3.77ab	10.4 $\pm$ 0.74bc	13.1 $\pm$ 1.25a
	Fe1.2	337 $\pm$ 19.6a	331 $\pm$ 45.2a	210 $\pm$ 23.6a	8.76 $\pm$ 1.60b	12.9 $\pm$ 1.11 ac	11.8 $\pm$ 0.56ab
	Fe2.0	331 $\pm$ 47.1a	335 $\pm$ 41.3a	219 $\pm$ 2.68a	9.39 $\pm$ 2.37b	15.9 $\pm$ 2.81 ac	13.4 $\pm$ 1.92a
Sb(V)	Fe0	117 $\pm$ 4.13c	191 $\pm$ 6.99b	156 $\pm$ 3.98ab	7.43 $\pm$ 0.95a	8.64 $\pm$ 0.80a	3.77 $\pm$ 0.38a
	Fe0.2	149 $\pm$ 24.3bc	200 $\pm$ 22.9b	154 $\pm$ 1.33ab	10.2 $\pm$ 1.22a	10.1 $\pm$ 1.06a	5.04 $\pm$ 0.10a
	Fe0.4	164 $\pm$ 4.91b	224 $\pm$ 15.3ab	149 $\pm$ 2.55b	8.00 $\pm$ 1.18a	6.70 $\pm$ 0.23a	3.13 $\pm$ 2.30a
	Fe0.7	184 $\pm$ 12.2ab	249 $\pm$ 18.8a	171 $\pm$ 9.15a	9.65 $\pm$ 0.47a	7.67 $\pm$ 0.16a	8.61 $\pm$ 1.33a
	Fe1.2	174 $\pm$ 5.01b	251 $\pm$ 4.56a	162 $\pm$ 4.11ab	8.49 $\pm$ 0.83a	7.98 $\pm$ 1.09a	5.42 $\pm$ 0.88a
	Fe2.0	213 $\pm$ 9.77a	250 $\pm$ 10.5a	153 $\pm$ 9.20ab	8.48 $\pm$ 2.06a	7.28 $\pm$ 0.31a	6.72 $\pm$ 0.45a
Analysis of Variance							
Cultivars (C)			$P<0.01$			$P<0.01$	
Fe			$P<0.01$			NS	
Sb Speciation (Sb)			$P<0.01$			$P<0.01$	
C $\times$ Fe			NS			$P<0.01$	
C $\times$ Sb			$P<0.01$			NS	
Fe $\times$ Sb			NS			NS	
C $\times$ Fe $\times$ Sb			NS			$P<0.05$	

when Sb(III) was supplied. Cultivars of NK got the highest Sb concentration in root with Sb(V) supplied (Table 2).

Antimony distribution in different plant components

Cultivars, Sb species and Fe^{2+} concentrations in the pretreatment solutions had significant effects on percentages of Sb in different plant components ($P < 0.01$, Table 3). Percentage of antimony in shoots was significantly lower than those in DCB extracts and roots, and accounted for up to 1–11% of the total Sb.

There was a significantly positive correlation between the amount of iron plaque and the percentages of Sb in DCB-extracts. However, the percentages of Sb in roots and shoots decreased when Fe^{2+} concentrations in pretreatment solution increased with Sb(III) or Sb(V) supplied for three cultivars (Table 3).

The percentage of Sb in DCB-extracts with Sb(V) supplied was lower than that with Sb(III) supplied. Whereas, percentages of Sb in roots and shoots with Sb(V) supplied were higher compared with those with Sb(III) supplied for three cultivars.

Cultivars had no effect on the Sb distribution in different plant components with Sb(V) supplied (Table 3). But different conclusions were found in Sb(III) treatment. The general orders of Sb distribution followed the ranking of $\text{YD} < \text{NK} < \text{JH}$ (Sb percentage in DCB-extracts), $\text{YD} > \text{NK} > \text{JH}$ (Sb percentage in roots) ($P < 0.01$). The percentages of Sb in shoot were not affected by cultivars treated with Sb(III).

Discussion

The DCB-Sb concentrations correlated significantly with the amounts of iron plaque on the root surfaces for the 3 cultivars that were exposed to different forms of antimony (Fig. 2; Fig. 3). This result was similar to As observed in the previous studies both in rice and other plant species (Greipsson 1995; Liu et al. 2004a, b). The highest Sb concentration in DCB-extracts was 2–4 times higher than the lowest concentration in DCB-extracts (FeO), which means Fe plaque can accumulate much more antimony on the rice root surface. The similar results were observed from other elements and plant species. Zhang et al. (1998) found

that iron plaque on root surfaces could accumulate Zn. Ye et al. (2001) reported that Cu concentrations in the DCB-extracts of *Typha latifolia* increased with increasing Fe plaque on the root surfaces. Nickel concentrations on the root surfaces of *Typha latifolia* with iron plaque were also significantly higher than those without iron plaque (Ye et al., 1997).

The DCB-extracted Sb on the root surfaces still correlated significantly with the antimony speciation. Sb(III) was more easily accumulated on the surfaces by the Fe plaque than Sb(V) (highest concentrations for YD: 838 mg kg^{-1} vs. 313 mg kg^{-1} ; NK: $1,832 \text{ mg kg}^{-1}$ vs. 374 mg kg^{-1} ; JH: $2,532 \text{ mg kg}^{-1}$ vs. 253 mg kg^{-1} , respectively) (Table 1). However, Liu et al. (2005) reported that arsenic concentrations in Fe plaque on the roots treated with arsenate were significantly higher than with arsenite. The possible reason was the different chemical properties of Sb(III) (supplied with $\text{KSbC}_4\text{H}_4\text{O}_7 \cdot 1/2\text{H}_2\text{O}$) and Sb(V) (supplied with KSb(OH)_6) in this study. Kamenar et al. (1969) reported that Sb(III) (supplied with $\text{KSbC}_4\text{H}_4\text{O}_7 \cdot 1/2\text{H}_2\text{O}$) exists as $[\text{Sb}_2(\text{C}_4\text{H}_2\text{O}_6)_2]^{2-}$ unit in aqueous solution with two negative charges. According to the pE-pH diagram for the Sb-O-H (Susan et al. 2010), the predominate species of antimonate is Sb(OH)_6^- at $\text{pH} > 3$ only with one negative charge. Therefore, the predominant species of Sb(III) and Sb(V) in solution are different from arsenate and arsenite which are H_2AsO_4^- and H_3AsO_3^0 at same pH range (Sadiq et al. 1983).

The Sb concentrations in the rice roots treated with Sb(III) were slightly higher than that with Sb(V) (Table 2), but this is not as significant as the Sb concentrations in the DCB-extracts (Table 1). The formation and amounts of iron plaque on rice roots had no significantly effect on the uptake of Sb(III) in roots (Table 2), but significantly increased the absorption of Sb(III) in roots (Table 2). This meant iron plaque was the source of Sb(V) transfer into the roots, but not for Sb(III). Liu et al. (2005) demonstrated that arsenic concentrations in roots were significantly lower when arsenate was supplied than when arsenite was supplied with Fe plaque. Liu et al. (2007) found that the formation of iron plaque on the roots of rice significantly decreased the Cd concentrations in roots. Zhang et al. (1998) suggested that Zn concentrations in rice roots increased and then decreased with increasing the amounts of Fe plaque on the root surfaces.

Table 3 Percentages of Sb in different components of three rice cultivars treated by different Sb species and the analysis of variance (means $\pm$ SE, $n=3$)

Sb Treatment	Fe Pretreatment	DCB-Sb%			Root-Sb%			Shoot-Sb%		
		YD	NK	JH	YD	NK	JH	YD	NK	JH
Sb(III)	Fe0	49.1 $\pm$ 1.2c	59.6 $\pm$ 2.3c	75.8 $\pm$ 2.1c	43.2 $\pm$ 0.6a	34.1 $\pm$ 1.7a	19.8 $\pm$ 1.3a	7.58 $\pm$ 1.1a	6.16 $\pm$ 0.6a	4.36 $\pm$ 0.9a
	Fe0.2	56.2 $\pm$ 2.7bc	71.3 $\pm$ 1.5b	84.7 $\pm$ 0.6b	38.7 $\pm$ 2.4 ac	25.7 $\pm$ 1.5b	13.4 $\pm$ 0.7b	5.05 $\pm$ 0.3bd	3.59 $\pm$ 0.4b	1.79 $\pm$ 0.1b
	Fe0.4	67.8 $\pm$ 2.5a	79.5 $\pm$ 1.8a	87.7 $\pm$ 0.6ab	26.6 $\pm$ 2.2b	18.7 $\pm$ 1.8bc	10.5 $\pm$ 0.4c	5.57 $\pm$ 0.8ab	1.66 $\pm$ 0.1c	1.68 $\pm$ 0.2b
	Fe0.7	62.5 $\pm$ 0.9ab	81.9 $\pm$ 2.4a	90.8 $\pm$ 0.5a	33.2 $\pm$ 1.1ab	15.7 $\pm$ 2.2c	7.67 $\pm$ 0.8 d	4.21 $\pm$ 0.9bc	2.27 $\pm$ 0.2c	2.25 $\pm$ 0.2b
	Fe1.2	68.9 $\pm$ 3.7a	82.2 $\pm$ 2.7a	90.7 $\pm$ 0.6a	28.7 $\pm$ 3.9bc	15.4 $\pm$ 2.7c	7.54 $\pm$ 0.6 d	2.34 $\pm$ 0.3c	2.31 $\pm$ 0.1c	1.75 $\pm$ 0.1b
	Fe2.0	62.6 $\pm$ 5.3ab	81.4 $\pm$ 2.6a	89.7 $\pm$ 0.6a	34.9 $\pm$ 6.1ab	15.7 $\pm$ 2.5c	8.25 $\pm$ 0.4 cd	2.97 $\pm$ 0.7 cd	2.79 $\pm$ 0.5bc	2.04 $\pm$ 0.2b
Sb(V)	Fe0	33.3 $\pm$ 2.7 d	30.7 $\pm$ 0.5 d	37.7 $\pm$ 1.7 d	54.9 $\pm$ 1.3a	59.1 $\pm$ 0.8a	56.2 $\pm$ 1.2a	11.6 $\pm$ 1.3a	10.1 $\pm$ 1.1a	6.01 $\pm$ 0.5a
	Fe0.2	43.5 $\pm$ 5.1c	40.4 $\pm$ 2.9c	45.7 $\pm$ 0.4c	45.4 $\pm$ 4.5b	49.8 $\pm$ 2.6b	48.7 $\pm$ 0.6b	11.0 $\pm$ 0.8a	9.73 $\pm$ 0.3a	6.18 $\pm$ 0.1a
	Fe0.4	47.9 $\pm$ 2.1bc	45.8 $\pm$ 1.7c	53.3 $\pm$ 1.8b	44.6 $\pm$ 1.3b	49.1 $\pm$ 1.9b	41.4 $\pm$ 0.1 cd	7.43 $\pm$ 1.1b	5.04 $\pm$ 0.7b	5.17 $\pm$ 1.7a
	Fe0.7	51.9 $\pm$ 2.1ab	51.5 $\pm$ 0.9b	53.4 $\pm$ 1.1b	40.8 $\pm$ 2.3bc	42.6 $\pm$ 0.7c	38.9 $\pm$ 0.1c	7.15 $\pm$ 0.3b	5.78 $\pm$ 0.3b	7.65 $\pm$ 1.1a
	Fe1.2	58.2 $\pm$ 0.7a	54.6 $\pm$ 2.3ab	50.9 $\pm$ 0.3b	35.7 $\pm$ 0.2c	40.3 $\pm$ 2.3c	43.7 $\pm$ 0.9 d	5.95 $\pm$ 0.5b	5.05 $\pm$ 0.6b	5.35 $\pm$ 0.8a
	Fe2.0	56.3 $\pm$ 0.9a	57.2 $\pm$ 1.7a	58.7 $\pm$ 2.3a	38.6 $\pm$ 2.8bc	38.2 $\pm$ 1.6c	35.5 $\pm$ 2.1e	5.01 $\pm$ 1.1b	4.53 $\pm$ 0.3b	5.65 $\pm$ 0.2a
Analysis of Variance										
Cultivars (C)			$P<0.01$			$P<0.01$			$P<0.01$	
Fe			$P<0.01$			$P<0.01$			$P<0.01$	
Sb Speciation (Sb)			$P<0.01$			$P<0.01$			$P<0.01$	
C $\times$ Fe			$P=0.05$			NS			NS	
C $\times$ Sb			$P<0.01$			$P<0.01$			$P<0.01$	
Fe $\times$ Sb			$P<0.05$			$P<0.05$			$P<0.01$	
C $\times$ Fe $\times$ Sb			NS			NS			NS	

The proportions of Sb in DCB-extracts and roots were significantly higher than those in shoots (Table 3), indicating that iron plaque and roots systems were the main barriers to antimony translocation. Fe plaque formation increased the percentage of antimony in DCB-extracts and roots, but decreased that in shoots (Table 3), indicating that Fe plaque may act as a 'buffer' to prevent Sb translocation from roots to shoots. Percentages of Sb in different components were also significantly affected by Sb species. The proportion of Sb in DCB-extracts treated with Sb(III) was much higher than that treated with Sb(V), whereas the percentages of Sb in roots and shoots showed the opposite trend. So we can conclude that Sb(III) translocation was more easily affected by iron plaque than Sb(V) translocation. Liu et al. (2004a, b) reported that more than 80% of the As existed in DCB-extracts with arsenate supplied, and As(V) was more easily accumulated in Fe plaque on the root surfaces than As(III). Liu et al. (2007) found that root systems absorbed much Cd and root tissue was the main barrier to Cd translocation, the proportion of Cd in roots accounted for approximate 65% of total Cd uptake.

Conclusions

Sb accumulated on the root surface were largely associated with the amounts of iron plaque when both Sb(III) and Sb(V) supplied. Fe plaque accumulates more Sb in Sb(III) treatment than Sb(V) exposure. Sb concentrations in roots and shoots with Sb(III) supplied were slightly higher than those treated with Sb(V). JH cultivar exhibited the highest Sb concentrations in DCB-extracts but the lowest Sb concentrations in shoots.

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