



# Can iron plaque affect Sb(III) and Sb(V) uptake by plants under hydroponic conditions

Ying Ji<sup>a,\*</sup>, Pierre Vollenweider<sup>b</sup>, Markus Lenz<sup>c,d</sup>, Rainer Schulin<sup>a</sup>, Susan Tandy<sup>a,\*</sup>

<sup>a</sup> Institute of Terrestrial Ecosystems, ETH Zürich, Universitätsstrass 16, 8092 Zürich, Switzerland

<sup>b</sup> Forest Dynamics, Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

<sup>c</sup> Institute for Ecopreneurship, University of Applied Sciences and Arts Northwestern Switzerland (FHNW), Grödenstrasse 40, 4132 Muttenz, Switzerland

<sup>d</sup> Department of Environmental Technology, Wageningen University, 6708 WG Wageningen, The Netherlands

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## ABSTRACT

Antimony (Sb) contamination of soils is of concern due to human activities such as recycling of Sb containing Pb acid batteries, shooting and mining. However Sb uptake by plants is poorly documented, especially when plants are growing on waterlogged soils and iron plaques form on their roots. The effect of iron plaques on Sb uptake has been investigated in rice, but not so far in other plants. Here, rye, ryegrass, wheat and meadow fescue were induced to form iron plaques and then exposed to antimonite (Sb(III)) or antimonate (Sb(V)) under hydroponic conditions. In the Sb(III) treatment, although iron plaques adsorbed Sb(III), this did not affect root and shoot Sb concentrations of plants. In the Sb(V) treatment, iron plaques adsorbed Sb(V) to a lesser extent than for Sb(III), although it was still significant in all plants but wheat. Iron treatments also significantly increased root Sb concentrations of fescue while they significantly decreased shoot Sb concentrations in rye, ryegrass and fescue. This may be due to other factors as well as antimony adsorption to iron plaques.

## 1. Introduction

Although defined by the United States EPA (U.S. EPA, 2014) as a priority pollutant since the 1970s, antimony (Sb) and its compounds have been less investigated than other priority pollutants such as arsenic (As). Antimony is not a naturally abundant element (0.2 mg kg<sup>-1</sup> in earth's crust), but due to anthropogenic activities and diffuse contamination its concentrations in the environment are increasing. World primary production of Sb reached 130,000 t in 2016, with China and Russia being the two major producers in the world (U.S. Geological Survey, 2017). The global Sb consumption is increasing due to its application in industrial processes, e.g. as a catalyst in Polyethylene terephthalate (PET) production, and as a hardening agent for lead used for many purposes among them ammunition and batteries. In countries including Switzerland, Canada, United States and Australia, which have large shooting range areas, Sb contamination of shooting range soils originating from the degradation of ammunition has become an environmental problem (Hockmann and Schulin, 2012; Laporte-Saumure et al., 2011; Sanderson et al., 2012; Van Vleet et al., 2011). In other countries such as China and Bolivia, mining and smelting activities are a major source of soils contamination by Sb (Fontúrbel et al., 2011; He et al., 2012).

In Switzerland many shooting range soils are temporarily or permanently waterlogged, which is also a worldwide phenomenon (Boyer, 1982; Dear et al., 2003; Hockmann, 2014; Merot et al., 1995). Waterlogging inhibits soil aeration and causes the redox potential of the soil solution to decrease and the oxidation state of Sb to be reduced (Wan et al., 2013). Normally, in aqueous environments Sb(V) is stable as Sb(OH)<sub>6</sub><sup>-</sup> under oxidizing conditions and Sb(III) as Sb(OH)<sub>3</sub> under reducing conditions (Hockmann and Schulin, 2012). Although some research has been carried out on the accumulation of different Sb species in plant roots, little is known about the mechanisms of Sb uptake by plants. As the molecular structure of Sb(OH)<sub>3</sub> mimics that of glycerol, a common notion is that the uptake of Sb(OH)<sub>3</sub> is mediated by transporters of the aquaglyceroporin subfamily in yeast, bacteria and human blood cells (Mukhopadhyay et al., 2014). In fact, the nodulin 26-like intrinsic protein NIP 1;1, which is an equivalent of aquaglyceroporins in plants, is able to transport Sb(III) in *Arabidopsis thaliana* (Kamiya and Fujiwara, 2009). Even less is known about the mechanisms of Sb(V) uptake by plants. It is hypothesized that Sb(V) is taken up by roots through the apoplastic pathway or through anion channels (Tschan et al., 2009b). A recent study found that chloride inhibited the uptake of Sb(V) in *Archillea wilhelmsii* which supports the anion channel hypothesis (Hajiani et al., 2017). However this may also be in conjunction

\* Corresponding authors.

E-mail addresses: [ying.ji@usys.ethz.ch](mailto:ying.ji@usys.ethz.ch) (Y. Ji), [susan.tandy@env.ethz.ch](mailto:susan.tandy@env.ethz.ch) (S. Tandy).

with the apoplastic pathway through the roots to the xylem, as another recently published study found that after uptake, Sb(V) remained in the same chemical form in the roots of ryegrass, but was partially converted to Sb(III) in the shoots which would require it entering the cells. (Ji et al., 2017). In a pot experiment, the roots of ryegrass and velvet grass grown in waterlogged soil accumulated more Sb than those in non-waterlogged soil, while Sb accumulation in the shoots of both plant species showed the opposite effect of waterlogging; velvet grass shoots accumulated less and ryegrass more Sb with than without waterlogging (Wan et al., 2013).

Waterlogging also influences the histological structure of plant roots. Under hypoxic conditions, the roots of some plants including sunflower, barley and maize are known to develop air-conducting intercellular spaces connecting the roots to the aerated above-ground organs, namely aerenchyma (Gunawardena et al., 2001; Kawase, 1979; Larsen et al., 1986). There are two types of aerenchyma, schizogenous, which are generated by cell separation and differential cell expansion and lysigenous, which arise from selective cell death. If the redox potential drops to values at which Fe (hydr)oxides undergo reductive dissolution, high concentrations of  $\text{Fe}^{2+}$  can develop in the soil solution. Under such conditions,  $\text{O}_2$  supply through aerenchyma can cause a sharp gradient in redox potential around the roots, and thereby through the oxidization of dissolved Fe, to the precipitation of  $\text{Fe}^{\text{III}}$ (hydr)oxides, so called “iron plaques” on the roots (Liu et al., 2006; Taylor et al., 1984). Iron plaques can retain metals such as Ni, Cu, Zn and Mn on the roots of various wetland plants (Jiang et al., 2009; Otte et al., 1989; Taylor and Crowder, 1983). Iron plaques on rice roots can also adsorb As (Liu et al., 2004; Seyfferth et al., 2010) and Sb (Cui et al., 2015; Huang et al., 2012; Ren et al., 2014). This effect is associated with decreased uptake of these metal(oids). Thus, Ren et al. (2014) concluded that iron plaques decreased Sb(V) accumulation in rice roots. However, the link between the two effects, i.e. iron plaque formation and reduced Sb uptake, is not sufficiently clear. Huang et al. (2012) reported that iron plaque formation was associated with decreased shoot Sb concentrations, but did not affect root Sb concentrations of rice plants exposed to Sb(III) in nutrient solutions. The same authors also observed an increase in root Sb, with no change in shoot Sb in rice plants exposed to Sb(V). Based on Cui et al. (2015) iron plaques inhibited uptake of both Sb(III) and Sb(V) in rice roots. The inconsistency in one plant species, rice, indicates that the role of iron plaques in plant uptake of Sb is complicated and might differ depending on variety of plant species and growth conditions. Adsorption to  $\text{Fe}^{\text{III}}$  (hydr)oxides differs between Sb(III) and Sb(V) due to their different charge and molecular structure of their hydrolyzed form. Adsorption of the oxyanion  $\text{Sb}(\text{OH})_6^-$  on charged surfaces of minerals such as (hydr)oxides is strongly pH dependent. In comparison, adsorption of the neutral molecule  $\text{Sb}(\text{OH})_3$  is much less influenced by pH (Leuz et al., 2006; Qi and Pichler, 2016). Furthermore, corresponding to their different molecular structures,  $\text{Sb}(\text{OH})_6^-$  forms bidentate edge-sharing surface complexes on iron hydroxides such as goethite, while  $\text{Sb}(\text{OH})_3$  forms bidentate corner-sharing complexes (Scheinost et al., 2006).

So far, studies designed to investigate the potential influence of iron plaques and Sb uptake looked at only one plant species, i.e. rice. However, the roots of other agricultural plants, including pasture grasses, may experience periods of exposure to waterlogging and respond with aerenchyma and iron plaque formation. Grassland vegetation exposed to waterlogging is particularly common in Swiss shooting ranges which are often used for forage production and grazing when no shooting activity is taking place. Motivated by the question of how this would affect fodder quality with respect to Sb accumulation, we here investigated the potential role of iron plaques on Sb accumulation by grasses commonly grown in these ranges and exposed to waterlogging as well as some related cereals that maybe found growing in agricultural soil around mines in China and Russia. The study started out from the hypotheses: 1) Aerenchyma and iron plaque formation are responses to waterlogging that are frequent among agricultural grasses

that are not specifically adapted to wetland conditions. 2) Iron plaques act as a barrier preventing Sb from entering roots. 3) The effects of iron plaques on the uptake of Sb(III) and Sb(V) are different, as they have different affinities for Fe (hydr)oxides.

## 2. Materials and methods

All chemicals used were of analytical or superpure grade. Nanopure water (18 M $\Omega$  cm) was used for preparing stock solutions. All plastic and glass containers were acid washed before use.

### 2.1. Pre-experiments

The following 8 commonly cultivated agricultural plants were chosen for screening, some on the basis of previous research (Tschan et al., 2008; Tschan et al., 2009a; Wan et al., 2013): ryegrass (*Lolium perenne* L. Calibra), meadow fescue (*Festuca pratensis* Huds. Preval), velvet grass (*Holcus lanatus* L.), rice (*Oryza sativa* L. Nipponbare), wheat (*Triticum aestivum* L. Sella), barley (*Hordeum vulgare* L. Ascona), rye (*Secale cereale* L. Palazzo) and sunflower (*Helianthus annuus* L. Iregi). Ryegrass, meadow fescue and velvet grass are common grasses in Swiss agricultural grasslands, including shooting ranges. Wheat, barley, rye and rice were included for comparison and because they are widely cultivated as cereals for food production. Rice in particular was included for comparison with previous works on iron plaque formation and Sb uptake. Sunflower was included as dicotyledonous plant species in the list of candidate plants selected for screening to compare aerenchyma formation in monocots and dicots.

#### 2.1.1. Screening for Sb uptake

Two Sb uptake experiments were performed for screening candidate plants for Sb uptake. The first experiment was carried out with ryegrass, rice, wheat, barley, rye and sunflower. The second experiment was carried out with four plant species: meadow fescue and velvet grass (new species) and ryegrass and rice (controls from the first experiment). The seeds were rinsed with 10%  $\text{H}_2\text{O}_2$  for 15 min, then washed with nanopure water and germinated on moist rolled tissue. After germination, all seedlings (four replicates per species) were transferred to opaque 1 L bottles filled with continuously aerated 20% Hoagland nutrient solution, buffered with 2-(*N*-morpholino)ethanesulfonic acid (MES) at pH 6, in a climate chamber. The climate chamber had a daily photo period of 16 h at 22 °C with 230  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photon flux and a daily night period of 8 h at 16 °C. The plants were grown under these conditions for four weeks, and the nutrient solutions were changed twice weekly during this time. After 4 weeks, 1 mg  $\text{L}^{-1}$  Sb(III) or Sb(V) was added to the nutrient solutions for 8 days. This level of Sb exposure was based on the research of Tschan et al. (2009a) and Vaculikova et al. (2016), which ensures plants accumulate enough Sb to enable analysis without reaching toxic levels. The Sb(V) stock solution was prepared by dissolving  $\text{KSbOH}_6$ , the Sb(III) stock solution from a solution of  $\text{Sb}_2\text{O}_3$  in 2 M HCl (Merck Millipore). Both stock solutions had a nominal concentration of 1000 mg  $\text{L}^{-1}$ . The same amount of HCl as in the Sb(III) solution was added to Sb(V) solution, and the pH was adjusted by 2 M KOH to pH 6. The nutrient solutions containing Sb(III) were not aerated, while those containing Sb(V) were aerated during the 8 days. The solutions were changed every two days and sampled before plants were introduced and after 2 days of plant growth, in order to analyze total Sb concentrations and Sb speciation. At harvest plants were separated into shoots and roots, rinsed in deionized and nanopure water, and then dried at 60 °C for 3 days.

#### 2.1.2. Screening for aerenchyma formation

Seedlings of all 8 candidate plant species were grown under aerated hydroponic conditions as described in the previous section. After 4 weeks, root segments of about 1 cm length were sampled from adventitious roots (1–2 cm away from the root apex). Then the samples

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