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The role of arbuscular mycorrhizal fungi in plant uptake, fractions, and speciation of antimony

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ABSTRACT

Microorganisms play an important role in the biogeochemical cycle of antimony (Sb). However, specific microorganisms have not yet been identified that mediate Sb transport and fate in soil-plant systems. Arbuscular mycorrhizal fungi (AMF) form a mutualistic symbiosis with roots of over 90% of terrestrial plants. In the present study, we investigated the inoculation effects of *Funneliformis mosseae*, an AM fungus, on Sb accumulation and speciation in *Cynodon dactylon* (Bermuda grass) using Sb(V) spiked soil at 0, 500, and 1000 mg kg^{-1} treatment levels. Results indicated plant biomass was significantly increased by the AMF symbiosis. Compared to un-inoculated controls, mycorrhizal colonization significantly increased shoot and root Sb concentrations under all Sb treatment levels. Bioconcentration (BCF) and translocation (TF) factors were elevated by mycorrhizal colonization. Mycorrhizal colonization significantly increased exchangeable Sb concentrations and decreased carbonate-associated Sb in the rhizosphere under all Sb treatment levels. Moreover, Sb(III) percentages relative to total Sb were significantly lower in mycorrhizal plants. These results suggested AMF likely inhibited Sb(V) to Sb(III) reduction processes, and thereby alleviated Sb toxicity in host plants. These findings demonstrated AMF serve an important role in Sb transport and fate in soil–plant systems.

1. Introduction

Antimony (Sb) is a toxic element and excessive exposure can result in various human diseases, including cancer, cardiovascular, liver, and respiratory diseases (WHO, 2003). Sb and its compounds are listed as priority pollutants by the US Environmental Protection Agency (US EPA, 1979) and the European Union (Filella et al., 2002). Sb soil pollution resulting from mining and manufacturing practices have become increasingly problematic due to the widespread industrial use of this metalloid (He, 2007; Wilson et al., 2010). A potential human and animal Sb exposure pathway is via food and feed, respectively as antimony is transported by plant roots from soil (Liu et al., 2009; Wu et al., 2011). Due to Sb toxicity and its current pollution patterns, many countries are currently concerned about Sb pollution (Flynn et al., 2003; He, 2007; Maher, 2009; Wilson et al., 2004).

Sb primarily occurs as a co-contaminant to more toxic elements, including arsenic (As) or lead (Pb), therefore research into its biogeochemistry has previously been neglected. Antimonite Sb(III) and antimonate Sb(V) are common environmental Sb species. Studies reported plants readily uptake and translocate these two Sb species via roots to shoots from soil, although Sb(III) and Sb(V) are not essential plant elements (Telford et al., 2009; Tschan et al., 2009; Qi et al., 2011). Plant Sb uptake and assimilation mechanisms have drawn much attention and remain elusive (Tschan et al., 2009). Sb and As are chemical analogs; however plant uptake and translocation differences exist between the two elements. Kamiya and Fujiwara (2009) found Sb and As were sister elements and Sb(III) entered plants via As(III) transporters. However, Sb(V) plant uptake mechanisms remain unclear. Asher and Reay (1979) reported phosphate transporters facilitated As(V) plant entry, but evidence indicated plant Sb(V) did not occur via the same route. For example, Tschan et al. (2008) showed As(V) had no effect on Sb(V) uptake in *Zea mays* and *Helianthus annus* under phosphate treatments.

Arbuscular mycorrhizal fungi (AMF) are soil fungi, which form mutualistic symbioses with over 90% of terrestrial plant roots

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(Smith and Read, 2008). Studies demonstrated arbuscular mycorrhizal symbioses can influence metal accumulation and speciation in host plants (Marques et al., 2006; Zhang et al., 2015). Due to its increased toxicity, the relationship between As and AMF has been reported frequently. Smith et al. (2010) found arbuscular mycorrhizas naturally occur in As-contaminated soils and mycorrhizal inoculation improved plant As tolerance (Dong et al., 2008; Leung et al., 2013). More importantly, AMF directly mediated plant As uptake under controlled conditions (Christophersen et al., 2009). In addition, AM symbioses alleviated As(V) toxicity by mitigating oxidative stress (Yu et al., 2009; Garg and Singla, 2012) and influencing different As species distributions in plants (Yu et al., 2009; Chen et al., 2012).

We previously found most plants grown in Sb mine areas developed symbiotic associations with AMF under natural conditions (Wei et al., 2015a,b). Moreover, Sb plant concentrations were significantly positively correlated with mycorrhizal colonization ($P < 0.01$)(Wei et al., 2015a,b) .Based on the analysis above, we inferred AMF might also play an important role in plant root Sb uptake and translocation from soil. However, direct evidence for the role of AMF in Sb transport and fate in host plants remains unavailable. The aim of the present study was to examine Sb behavior in soil–plant systems following inoculation with the arbuscular mycorrhizal fungus *Funneliformis mosseae*. *Cynodon dactylon* (Bermuda grass) was used as the model plant and the soil was spiked with Sb(V) at different concentrations. Plant Sb uptake and distribution were characterized and compared between mycorrhizal and non-mycorrhizal treatments to understand AMF inoculation contribution to plant Sb uptake and translocation. The overall objectives of our study were to explain the role of symbiotic microorganisms in plant Sb uptake.

2. Materials and methods

2.1. Host plants

Bermuda grass (*Cynodon dactylon* (L.) Pers.) seeds were surface sterilized in 10% (v/v) H_2O_2 solution for 10 min, then immersed in deionized water for 10 h. Seeds were subsequently pre-germinated on moist filter paper for approximately 48 h at 27 °C until radicles emerged. Seeds were selected for radicle uniformity prior to sowing.

2.2. AMF inoculums

The Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry provided the AMF *Funneliformis mosseae* (Nicol. & Gerd.) Schüßler and Walker (BGC BJ05A). The fungus was propagated in pot cultures with Bermuda grass grown in sandy soil for 10 wk. Inoculum from pot cultures was a mixture of spores, mycelium, sandy soil, and root fragments.

2.3. Cultivation media

Calcareous soil naturally occurring was collected from the courtyard at the Chinese Research Academy of Environmental Science in Beijing. The experimental soil was air-dried, ground, and passed through a 2-mm sieve. The soil exhibited the following properties (on a dry weight soil basis): pH 7.8 (1:2.5 soil to water); 0.43% organic matter; 3.9 mg kg^{-1} 0.5 M $NaHCO_3$-extractable `P; 60.4 mg kg^{-1} 1 M NH_4OAc-extractable K; and 2.2 mg kg^{-1} total antimony. The experimental soil was sterilized by r-ray (20 kGy, 10 MeV electron beam) and received mineral nutrients of 300 mg kg^{-1} N (NH_4NO_3), 50 mg kg^{-1} P (KH_2PO_4), and 200 mg kg^{-1} K (K_2SO_4) as basal fertilizers before use.

2.4. Experimental procedure

Round plastic pots, which accommodated 3 kg soil, were used to cultivate test plants. Antimony was added as Sb(V) in solution (potassium hexahydroxoantimonate ($KSb(OH)_6$)) and mixed with soil by shaking for 24 h to obtain artificially homogeneous contaminated soils at concentrations of 0, 500, and 1000 mg kg^{-1}, respectively. Soils were equilibrated for a 4 wk period by undergoing four cycles of saturation with deionized water and air-dried in a greenhouse. Pots received a mixture of 2 kg soil and 50 g *F. mosseae* inoculum for mycorrhizal treatments or sterilized inoculum plus 15 mL of inoculum washings, filtered through a 37 μm filter paper for non-mycorrhizal controls (to provide similar soil microflora, with the exception of the mycorrhizal fungus). Two inoculation treatments were combined with three Sb(V) addition levels, resulting in six total treatments. Each treatment had three replicates, resulting in a total of 18 pots in a two-factor completely random design.

Ten seeds with emergent radicals were transplanted into each pot (18 total pots) and thinned to six seedlings per pot one week after seedling emergence. Plants were grown under controlled greenhouse conditions with a daytime photoperiod of 14 h at a light intensity of 250 μmol $m^{-2}s^{-1}$ provided by supplementary illumination. Day- and nighttime temperatures were respectively maintained at 25 °C and 18 °C. De-ionized water was added as required to maintain moisture content at ~50% of the water holding capacity determined by regular weighing.

2.5. Harvest and chemical analysis

Plants were harvested following 10 weeks of growth. Plant shoots and roots were harvested separately. Root samples were first carefully washed with tap water to remove adhering soil particles and subsequently rinsed in ice-cold phosphate buffer solution, which included 1 mM K_2HPO_4, 5 mM MES, and 0.5 mM Ca $(NO_3)_2$ for 20 min to remove Sb in root apoplasts. Roots and shoots were then carefully washed with de-ionized water, blotted dry, and weighed. Sub-samples of fresh roots were collected to determine AMF colonization. The remaining samples were frozen in liquid nitrogen and dry weights were recorded after freeze-drying for 72 h. Plant roots were shaken lightly to remove most soil and the remaining adhering soil particles were collected with hairbrush as rhizosphere soils. Rhizosphere soils were sampled from each pot for simultaneous Sb fraction determination.

Fresh root sub-samples were collected from each pot, cleared in 10% KOH, and stained with Trypan blue. Percentage root colonization was determined based on McGonigle et al. (1990).

Total acid digestion of plant samples was conducted in a closed microwave digestion device (MarsXpress, CEM) using concentrated HNO_3 (65–68%), HCl (36–38%), and HF (>40%) at 6: 3: 1 with 0.2 g sample and 6 mL acid (190 °C, 15 min). After cooling to room temperature, the samples were filtered and diluted to 50 mL with ultrapure water. Total Sb was digested using this method. Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, 7500c Agilent, USA) was employed to determine total Sb concentrations, and the limit of determination was 0.15 μg L^{-1}. A soil reference material (GBW07309 from China) and a plant reference material (GBW 10048 from China) were analyzed to determine the reliability of the method. The Sb concentration

that was found (0.94 ± 0.03 mg kg^{-1} and 0.056 mg kg^{-1}) agreed well with the reference concentration (0.93 ± 0.32 mg kg^{-1} and 0.053 ± 0.003 mg kg^{-1}).

The sequential extraction procedure followed Tessier et al. (1979). The extraction used 0.2 g air-dried rhizosphere soils and four fractions were extracted in the following sequence: exchangeable (1 M $MgCl_2$); carbonate-associated (1 M NaAc); amorphous