



Influence of arbuscular mycorrhizal fungi on antimony phyto-uptake and compartmentation in vegetables cultivated in urban gardens



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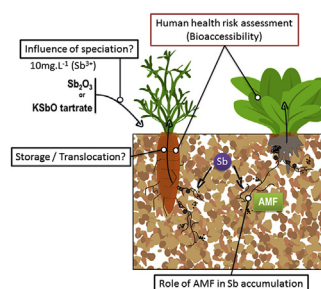
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HIGHLIGHTS

- AMF can increase Sb accumulation and its human bioaccessibility in edible organs.
- Impact of AMF on Sb translocation to aerial part is Sb species dependent.
- It's the first study on the role of *Rhizophagus irregularis* in Sb phytoavailability.

GRAPHICAL ABSTRACT



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ABSTRACT

1. Context: Urban areas are often contaminated with various forms of persistent metal (loid) and emerging contaminants such as antimony (Sb). Thus, in the context of urban agriculture where sustainable practices such as biofertilizers application (arbuscular mycorrhizal fungi, AMF) could improve nutrient transfer from the soil to the vegetables, the effect of AMF on metal (loid) mobility and human bioaccessibility is still poorly known.

2. Methods: The role of AMF in Sb uptake by lettuce and carrot grown in artificial substrate spiked with different Sb chemical species was investigated. Plants were grown under hydroponic conditions and half of the treatments received a concentrated spore solution to obtain mycorrhized and non-mycorrhized plants. Three weeks before harvest, plants were exposed to 10 mg.L^{-1} of either Sb_2O_3 or KSbO-tartrate (KSb).

3. Results: The presence of AMF significantly increased its accumulation in carrots (all organs) with higher accumulation in roots. In lettuce, accumulation appeared to be dependent on the Sb chemical species. Moreover, it was observed for the first time that AMF changed the human bioaccessible fraction of Sb in edible organs.

4. Implications: The present results highlight a possible risk of Sb transfer from soil to edible plants cultivated in soil naturally containing AMF propagules, or when AMF are added as biofertilizers. After validating the influence of soil environment and AMF on Sb behavior in the field, these results should be considered in health risk assessments.

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1. Introduction

Antimony (Sb) is a naturally occurring trace metalloid (TM) in soil (He et al., 2012). Its concentration is usually low in the environment, with about 0.2 ppm in the continental crust (Filella et al., 2002) and a background concentration in uncontaminated soil between <0.3 and 8.4 mg kg⁻¹ mostly due to volcanic activity (Hammel et al., 2000). But, its increasing use in industrial processes such as brake linings and fire retardants, but also its mining and smelting generate abnormally high levels of this toxic compound in the vicinity of (peri)urban areas (Adriano, 1986; He, 2007; Wiseman et al., 2013). The toxicity of Sb to humans has been investigated using various protocols (Gebel et al., 1998; Winship, 1987), and it is now considered a priority pollutant in many countries (Filella et al., 2007; WHO, 2008). However, legal thresholds are still scattered, with heterogeneous norms for water quality from 5 to 20 µg.L⁻¹ depending on the country (WHO, 2008) and none for soil and vegetable.

Antimony mobility in soil has been strongly documented over the last decades and it is usually considered as low due to: (1) partitioning to metal (hydr)oxides of Al, Fe, and Mn (Rakshit et al., 2011; Xu et al., 2011), (2) precipitation with secondary minerals (Diemar et al., 2009; Roper et al., 2012), and (3) interactions with humic acids and clay mineral (Ilgen and Trainor, 2012; Tighe et al., 2005; Xi et al., 2010).

Antimony has no physiological role in living organisms, but it can accumulate in the food chain, especially in plants (Feng et al., 2013; Pierart et al., 2015). A survey of its concentration in edible plants at the global scale highlighted that its prevalence is currently increasing (ANSES, 2011). Thus there appears to be a potential health risk for urban gardeners.

Plants have developed various intra or extracellular mechanisms to adapt to and overcome toxic metal (loid) exposure (Amir et al., 2014). In many cases, symbionts such as arbuscular mycorrhizal fungi (AMF) are involved (Joner et al., 2000).

The mechanisms required in TM mobility in the context of the AMF-plant interaction have been widely described (Ferrol et al., 2016; Joner et al., 2000; Leyval et al., 2002). These fungal pathways generally entail either an increased accumulation of the metal (loid) which allows phytoextraction/remediation (Danh et al., 2014), or a phytoprotective role with a decrease of TM in the plants (Rangel et al., 2014). AMF can block TM outside the root system through either binding in hyphal walls or complexation in soil by excreting organic acids or proteins such as glomalin (González-Chávez et al., 2004). Once TM has entered the fungus, its stress is sometimes alleviated by its compartmentation in fungal vesicles (Turnau, 1998) and specific gene activation of oxidative stress alleviation metabolism (Zn transporters, metallothioneins, heat shock proteins, and glutathione S-transferase) both in the fungus (Hildebrandt et al., 2007) and in the host plant (Repetto et al., 2003). However, the mechanisms are highly complex and no universal pathways have been found yet.

To date, while many studies focused on metal (loid)s with major health and environmental risks (Pb, As ...), relatively few focused on the health risks of Sb in food and many fields of investigation remain to be clarified (Filella et al., 2007; Feng et al., 2013). Indeed, there is very little information on the relationship between Sb and AMF (Affholder et al., 2014; Pierart et al., 2015) and no data on the health consequences are available. Recently, Wei et al. (2016) studied the interaction between Bermuda grass (*Cynodon dactylon* L. Pers.) and *Funneliformis mosseae* (a commonly studied AMF for its ability to change TM mobility in soil and accumulation in plants). The authors showed that *F. mosseae* increased the exchangeable Sb fraction (1M MgCl₂ extract) and decreased the

carbonate-associated fraction (1M NaAc extract) in soil. The authors also found that this AMF species increased the Sb translocation factor (TF) from roots to shoots leading to a higher level of Sb in shoots of mycorrhized Bermuda grass. If a similar mechanism occurred in edible plants, it could present a health risk. However, further studies are still needed as the response in terms of phytoaccumulation is highly dependent of the fungal species in the symbiotic relationship and the plant considered (Pierart, 2016). For example, antimony has been shown to hyperaccumulate in the fruiting body of *Chalciporus piperatus*, an ectomycorrhizal fungus, up to 1400 mg kg⁻¹ (Borovička et al., 2006). The saprophytic fungus *Scopulariopsis brevicaulis* can also methylate and volatilize Sb to alleviate the stress induced by its presence (Andrewes et al., 1998).

However, to date there has been little agreement on which Sb species can be transformed in this type of metabolic pathway (Ainsworth et al., 1991), and none of the studies reviewed appear to have controlled for the effects of AMF on Sb methylation or volatilization. Furthermore, a recent discovery showed several fungi species (*Fusarium oxysporum* CZ-8F1, *Penicillium janthinellum* SM-12F4, and *Trichoderma asperellum*) can demethylate methylated arsenic over time, limiting therefore eventual detoxification through volatilization (Su et al., 2015). Bacterial methylation of antimony has also been found to occur in sewage sludge spiked with Sb (Wehmeier and Feldmann, 2005). Furthermore, Sb stress seems to be alleviated through activation of an Ars operon containing genes encoding efflux transporters and enzymes from the reductase family in *Escherichia coli* species (Diorio et al., 1995).

Our objective was therefore to investigate for the first time the role of AMF strains potentially tolerant to Sb stress (isolated from contaminated soils) in Sb transfer from soil to lettuce and carrot, two vegetables frequently grown in urban gardens with a potential risk of metal accumulation (Xiong et al., 2014).

2. Material and methods

2.1. Plant cultivation

POT PREPARATION. Two layers of wintering veil (Gerbaud®) were placed at the bottom of each 1.5L plastic pot (Ø 14 cm, Soparco®). Each pot was filled with 1.3 kg of sterile (20min, 121 °C) inert substrate (Sorbix US-Special G®, Supporting information, Table SI.1). Plants were watered by capillarity to limit spores and Sb lixiviation processes. Once every two weeks, water was replaced with 'low Phosphate' Long Ashton solution at 7.5 µM final P concentration (Appendices, Supporting information, Table SI.1).

TRANSPLANTATION. Organic lettuce (*Lactuca sativa* L. var. *Appia*) and carrot (*Daucus carota* L. var. *Rothild*,) were sown on sterilized Sorbix and grown under greenhouse conditions (artificial lights were turned on when the brightness < 2800 lx, 18/20 °C day/night - 50% relative humidity) for four weeks acclimation. Two plants were transplanted per pot and after 1 week acclimation the smallest one was removed. A fully randomized design was adopted to account for heterogeneity in greenhouse climatic conditions. The different treatments are summarized in Table 1.

MYCORRHIZATION. 10 ml of a spore suspension (350 spores. ml⁻¹) was spread directly underneath the substrate surface, near the root system. The spore solution was prepared by soil extraction and wet sieving (300 > 180 > 40 µm) from pots containing leeks cultivated in Sb contaminated soil (Pierart, 2016). Fungal species in the spore solution were identified by high throughput Illumina Miseq (GET-Plage, Toulouse) with the ITS2ngs primers (ITS3ngs4/ITS4ngs -CATCGATGAAGAACGTAG/TCCTSCGCTTATTGATATGC) (Tedorsoo et al., 2015). The communities identified with Mothur (Schloss et al., 2009) and Usearch softwares (Edgar, 2013) were mainly composed of a pool of Glomerales species (85.2%) including

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