Ecotoxicology and Environmental Safety 162 (2018) 400–407

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Impacts of silicon addition on arsenic fractionation in soils and arsenic speciation in *Panax notoginseng* planted in soils contaminated with high levels of arsenic

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ARTICLE INFO

Keywords:
Panax notoginseng
Silicon
Arsenic speciation
arsM gene
Arsenic fractionation

ABSTRACT

Arsenic (As) is a well-known carcinogenic substance whose biological toxicity in soils and plants depends on its concentration and chemical forms. Silicon (Si) generally can alleviate biotic and abiotic stresses, including As stress. However, its effects vary depending on As chemical form, plant species and other factors. A pot experiment was performed to investigate the effects of Si addition on the content and forms of As in red soil and its uptake, transport and speciation in *Panax notoginseng*. The results showed that additions of 25 and 75 mg kg^{-1} of Si both significantly decreased the concentrations of water-soluble As and exchangeable As in soil and therefore decreased the bioavailability of soil As. However, the As uptake by *Panax notoginseng* (PN) was increased, which resulted in increases in As concentration by 18.5% and 2.3% in roots and by 56.7% and 58.3% in shoots, respectively, when compared with the control. Arsenate (As(V)) was the dominant As species in all the treatment soils (99.8–100%), whereas arsenite (As(III)) was prevalent in plant roots (75.2–92.4%), shoots (74.1–87.9%) and leaves (73.9–84.3%). Si addition (25 and 75 mg kg^{-1}) significantly increased As(III) concentration in roots by 167.5% and 83.3%, respectively. Monomethylarsonic acid (MMA) was the only detected methylated As but at low concentrations (0.01–0.29 mg kg^{-1}) and only in PN leaves. Si addition (25 and 75 mg kg^{-1}) significantly increased the copy number of the arsenite methyltransferase (*arsM*) gene by 31.0% and 47.2% but did not increase the methylated As species content in PN leaves. The detected copy number of the *arsM* gene did not represent the capacity of soil to methylate As, and the sources of MMA in leaves need to be explored in further research.

1. Introduction

Inorganic arsenic (As) is a carcinogenic substance that is ubiquitous in the environment (WHO, 2001). Millions of people have suffered from As exposure in South and Southeast Asia, South America and elsewhere (Brammer and Ravenscroft, 2009; Nordstrom, 2002). Excessive intake of As by crop plants may cause a product safety problem (Zhao et al., 2009) and harm human health through dietary consumption. The biological toxicity of As in soils and plants depends on the concentration and chemical forms of As (e.g., As speciation and As fractions in soil). The toxicity of inorganic As species (e.g., arsenite As(III) and arsenate As(V)) is generally greater than that of organic As (e.g., monomethylarsonic acid (MMA) and dimethylarsonic acid (DMA)). Among the inorganic As species, As(III) is more toxic than As(V), and determining As speciation in soil-plant systems is therefore important (Zhao et al., 2010). Many soil microorganisms can methylate As; bacteria, fungi and some algae in soils contain the arsenite methyltransferase (*arsM*) enzyme, which catalyzes inorganic arsenic methylation (Liu et al., 2011; Marapakala et al., 2012; Qin et al., 2009, 2006; Zhang et al., 2013). The *arsM* gene, which encodes arsenite methyltransferase, has been identified in bacteria (Qin et al., 2006), but in higher plants, no *arsM* gene has been cloned. The bioavailability and mobility of As in soil are closely related to the As fractions in soil. The use of soil sequential extraction procedures (SEPs) can differentiate the different extractable fractions of soil As; in addition, SEPs are useful in predicting changes in the mobility of As in soil as a result of soil amendments such as the addition of silicon (Si) (Wenzel et al., 2001).

Si is the second most abundant element in soil, and the concentrations of Si in plant tissues range from 0.1% to 10% of the plant dry weight (Ma, 2004). Generally, Si can enhance plant growth and alleviate various biotic and abiotic stresses, including As stress (Adrees et al., 2015; Ma, 2004). Studies have found that Si increases As levels in

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https://doi.org/10.1016/j.ecoenv.2018.07.015
Received 3 April 2018; Received in revised form 1 July 2018; Accepted 3 July 2018

soil solutions and inhibits As accumulation by rice (Fleck et al., 2013; Geng et al., 2018; Li et al., 2009; Seyfferth and Fendorf, 2012; Wu et al., 2016). Arsenite (As(III)) and silicic acid are similarly sized tetrahedral molecules with high pKa values (9.2 and 9.3 for arsenous acid and silicic acid, respectively) (Zhao et al., 2009). These two species compete for soil colloid sites, which results in increased As levels in soil solutions, as well as for transporters that allow entry into roots, decreasing As uptake by rice. Transformation of As from one speciation to another results in increased complexity. It was reported that Si addition to a cultivation medium inhibited the uptake of As(V) by rice seedlings (Guo et al., 2005). Si addition, however, increased DMA concentrations by 39% in rice grains (Li et al., 2009). Limmer et al. (2018) reported that Si treatments shifted As speciation in rice grain toward organic As, thereby decreasing the As toxicity to humans. In addition, Si's effect on As uptake and translocation is thought to be related to plant species. Marmiroli et al. (2014) reported that Si decreased the uptake and translocation of both As(III) and As(V) in tomato cultivar (cv.) Gladis, whereas Si supplementation was effective in decreasing As uptake and translocation in cv. Frigio when As(III) was provided; however, uptake increased when As(V) was provided, indicating that As uptake and translocation depend on the specific tomato cultivar. Studying the effects of Si on As uptake and transport in different species of higher plants is therefore important.

Panax notoginseng (Burk.) F.H. Chen (PN) is a traditional Chinese medicine that is widely used in China, Japan, Korea, and other countries (Zhu et al., 2017). PN promotes blood circulation, lowers blood pressure, improves resistance against thrombus formation, protects nerves, and improves health (Ng, 2006). PN production in Wenshan autonomous prefecture (Yunnan Province, China), the major PN-producing region, accounts for more than 90% of PN production worldwide (Yan et al., 2013). The high background values of soil As, frequent mining activities and large-scale use of As-containing pesticides (Ramirez-Andreotta et al., 2013; Williams et al., 2007) have resulted in increased As accumulation in PN. High As concentrations (517 mg kg^{-1}) in soil have been shown to decrease PN biomass and increase As accumulation in PN drugs, consequently threatening human health (Liu et al., 2018; Yan et al., 2012). Although several studies (Lin et al., 2015; Yan et al., 2012; Yan et al., 2013) did not find methylated As species in PN tissues in pot experiments, Ma et al. (2016) detected methylated As species in PN tissues under field conditions and speculated that As methylation may occur in PN. Methylated As species were also recently detected in PN roots in pot experiments (Zeng et al., 2016; Zhu et al., 2017). A study of the possible source of methylated As species in PN suggested that methylated As species were most likely from soil (Zhu et al., 2017). More experiments should therefore be performed to investigate whether methylated As species in PN plants are related to plants or soils.

The objectives of this study were to evaluate the effect of Si on As uptake by PN via examining As, Si, P concentrations in PN and the As fraction in soils; to explore the possible mechanism by which Si affects As speciation in PN by detecting different As species in PN; and to investigate the effect of Si addition on methylated As species in PN by analyzing the correlations between the concentration of methylated As species in PN and the abundance of the *arsM* gene in rhizosphere soils via a pot experiment.

2. Materials and methods

2.1. Pot experiment

Pot experiments were performed at the Wenshan experimental station (Yunnan, China, 24.72°N, 104.39°E, 1468 m above sea level) beginning in December 2015. As-contaminated soil (total As: 3849.9 mg kg^{-1}) was obtained from a site (0–20 cm soil depth) near the Wenshan Arsenic Factory field. Red soil (total As: 33.5 mg kg^{-1}) obtained from the cultivation base was added to the As-contaminated soil to obtain the experimental soil (total As: 386.7 mg kg^{-1}) in which PN was grown. All the soils used in the experiments were air-dried, sieved to < 0.75 cm and thoroughly mixed and incorporated. For the properties of the soils, see Table S1. Potassium silicate (K_2SiO_3) was dissolved in deionized water and then sprayed into the experimental soil, and the mixture was homogenized. Three treatments were established with Si concentrations of 0 (Control (CK)), 25 (Si-25) and 75 (Si-75) mg kg^{-1} soil. The soils were then placed in 10-L plastic pots, with 7.5 kg of soil per pot, and watered once every three days. Ten replicates were performed for each treatment. After the added K_2SiO_3 had stabilized in the soil for one month, four annual PN seedlings were transplanted into each pot. The pots were arranged in a randomized block design in a greenhouse. The soil moisture was maintained at 45% of field capacity, and the average annual temperature was 16.6 °C.

2.2. Sample collection and preparation

Plant and rhizosphere soil samples were collected 150 d after annual PN seedlings were transplanted. The plant samples were washed thoroughly to remove the soil and were finally rinsed twice with deionized water. Each plant sample was divided into root, shoot, and leaves. Stones and other impurities in the soil samples were removed by hand. Some aliquots of plant samples were oven-dried at 55 °C, whereas others were freeze-dried in a freeze dryer (CHRIST, Germany) for approximately 48 h. Some aliquots of soil samples were air-dried, and others were freeze-dried in a freeze dryer (CHRIST, Germany) for approximately 48 h. The oven-dried plant samples and the air-dried soil samples were ground using a grinding mill (ZM200 pulverisette 14, Germany) for determining the plant total As, Si and P; the soil total As; the soil pH and the soil As fractions. The freeze-dried plant and soil samples were pounded in a mortar in liquid nitrogen to produce a fine powder for As species analysis. Soil solutions were collected using Rhizon samplers (Soil Moisture Corp.), which were connected with a 50-mL pre-evacuated injector 24 h after irrigation. Each 10-mL aliquot of solution was supplemented with 0.1 mL of HCl to acidify the sample and thereby preserve the ability to detect pore water As, Si, and P contents. However, the concentrations of As and P were below their detectable limits.

2.3. Determination of total As in soil and total As, Si and P in plants

To determine the content of As in soil, we digested air-dried soil samples (0.1 g, Mettler Toledo AG 204, Switzerland) with a 5:2 (v/v) mixture of HNO_3 and HF in an automatic graphite digester (DigestLinc-ST60, China). After digestion was completed, the resulting solution was diluted to 25 mL for analysis. The oven-dried plant samples (0.2 g, Mettler Toledo AG 204, Switzerland) were digested with 2 mL of HNO_3 in a polytetrafluoroethylene tube and then placed into a stainless-steel jacket that was tightened and then heated at 200 °C for 4 h. The volume was adjusted to 10 mL after the sample had cooled. The Si in the plant and soil samples was extracted by alkali fusion using potassium hydroxide (KOH) and boric acid (HBO_3) (Mutsuga et al., 2011).

The total As, Si and P levels in PN roots and the cultivated soil were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (SPECTRO ARCOS EOP, Germany). For the main instrument working parameters, see Table S2. Quality control of the As analysis was performed using a blank reagent, standard reference soil (GBW07419) and the plant *Astragalus mongholicus* (GBW10028) (from the National Research Center for Standards in China). Analysis of the reference plant yielded the following values: As (mg kg^{-1}): 0.62 (certified value: 0.57 ± 0.05), P (mg kg^{-1}): 2114 (certified value: 2250 ± 120), and Si (%): 0.71 (certified value: 0.71). Analysis of the reference soil yielded the following values: As (mg kg^{-1}): 14.9 (certified value: 13.7 ± 1.2).