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Arsenic uptake by arugula (*Eruca vesicaria,* L.) cultivars as affected by phosphate availability

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HIGHLIGHTS

- arugula cultivars variably accumulated As from soils and nutrient solutions.
- High phosphate in nutrient solution strongly suppressed As uptake into shoots.
- Roots contained up to 400 times higher concentrations of As than shoots.
- Roots exposed to arsenate had greater sulfur concentrations.

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ABSTRACT

To assess the importance of variation among arugula (*Eruca vesicaria subsp. sativa*) cultivars in the ability to accumulate arsenic (As) in above-ground tissues, uptake of As by 16 cultivars was measured in the field and in hydroponic culture. In the field trial on soil contaminated by past pesticide use, As soil-plant uptake coefficients varied by a factor of 2.7 among different cultivars, approaching a value of one for the strongest accumulators. Compared to the field assay, hydroponically grown arugula accumulated much lower concentrations of As when nutrient solutions contained standard (high) concentrations of phosphate along with 1.0 mg L^{-1} As in the form of soluble arsenate. However, As accumulation was much greater in hydroponic culture using low-P nutrient solutions, an indication that phosphate strongly competed with arsenate for root uptake. Analysis of arugula roots after exposure to arsenate at 1.0 mg As L^{-1} and low phosphate revealed from 24 to 400 times greater As concentration in roots than tops, with S concentrations significantly greater in As-exposed than control roots. This indicated greater sulfate uptake by roots exposed to arsenate, and suggested that thiol-mediated As immobilization occurred in the roots which strongly restricted translocation to the tops.

1. Introduction

A number of plant species in the brassica genus are notable for their bioaccumulation of and tolerance to toxic metals and metalloids (Ebbs and Kochian, 1997; Grifoni et al., 2015; Karimi et al., 2009; Szczyglowska et al., 2011), and are therefore promising candidates for phytoremediation of soils. More specifically, some brassicas have unique abilities to accumulate and detoxify As, possibly by forming phytochelatin (PC) complexes (Karimi et al., 2009). Arsenate uptake occurs via root phosphate channels, so that addition of phosphate to hydroponic growth media or to crop irrigation water generally inhibits As accumulation by plants (Karimi et al., 2009; Meharg and Macnair, 1991; Pigna et al., 2010). Although arsenate uptake by plants is not believed to involve sulfate transporters, the sulfate status of plants can influence thiol levels and arsenate tolerance (El-Zohri et al., 2015) as sulfate uptake has a critical role in As detoxification in plants via thiol bonding of arsenite in antioxidant compounds (Grifoni et al., 2015; Mallick et al., 2013). Recent evidence that exposure to arsenite induces a sulfur starvation response in barley *(Hordeum vulgare)* seedling (Reid et al., 2013) is supportive of this mechanism, suggesting that greater sulfate uptake by roots would be a likely response to arsenate or arsenite exposure. The As hyperaccumulator, *Pteris vittata*, has enhanced sulfate uptake when exposed to As in the growth medium and produces higher levels of low-molecular weight thiols as a result (Watanabe et al., 2014).

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Non-hyperaccumulator plants exposed to As have also shown alteration of sulfur metabolism in roots to produce higher levels of phytochelatins (Ruiz-Torres et al., 2017). In addition, sulfur amendments to plant growth media have been shown to reduce As uptake and toxicity (Dixit et al., 2015; Yangyang and Guirong, 2014; Zhong et al., 2011). Consequently, the levels of both available phosphate and sulfate in soils need to be considered in assessing As uptake potential and toxicity in plants. Competition of arsenate uptake with that of other essential nutrients may also occur, although there has been little investigation on the interactions between As and micronutrients in plants challenged by high available As.

Among the brassicas, arugula (*Eruca vesicaria subsp. sativa*) appears to be a strong As accumulator based on limited evidence. Specifically, Stilwell et al. (2010) found arugula grown in As-contaminated soil to contain as much as 60 mg kg^{-1} As with a soil-plant uptake factor somewhat greater than 1.0, much higher than that of lettuce or basil. The As contaminant in this case was chromated copper arsenate (CCA), a relatively soluble form of As that can leach out of CCA-treated wood into garden beds. In contrast, Villatoro-Pulido et al. (2013) found much lower As concentrations and soil-plant uptake factors (0.005–0.03) for several arugula accessions grown in As-contaminated soil collected near a pyrite mine. To our knowledge, the only published studies to assess As uptake by arugula from old orchard sites where soils were historically contaminated by Pb arsenate insecticide use are those by Lim and McBride (2015) and Lim et al. (2017). In comparing observations from the limited studies involving arugula, it is clear that the original form of the As contamination in soil is a critical factor influencing long-term bioavailability and plant uptake, and that arugula is a stronger As accumulator than many other vegetables (although not a hyperaccumulator) that may be useful in assays to assess exposure risk for contaminated sites. Therefore, the present study was undertaken to address three key questions about As bioaccumulation in arugula:

1. How much variation in potential for As bioaccumulation exists among arugula cultivars from various regions of the world?
2. To what extent does phosphate availability impact As uptake and transfer into the above-ground tissues of arugula?
3. What effect does As exposure have on root uptake and transfer of macronutrients and trace elements into the tops of arugula plants?

2. Materials and methods

2.1. Field assay of arugula cultivars

Seeds of 16 arugula cultivars were obtained from the USDA-GRIN seed bank (see Table 1). About 10 seeds of each were planted in groupings in a small plot established at an old apple orchard in mid-May 2015. The total soil As and Pb in this plot were 191 and 1040 mg kg^{-1}, soil pH was 5.6, and soil organic matter was determined to be 8.1% by loss-on-ignition (Lim and McBride, 2015). In order to estimate the soluble and plant-available concentrations of As and P in the soil solution, duplicate 250 g samples of this soil were placed in Buchner funnels fitted with Whatman #42 paper filters, wetted to saturation with deionized water, and allowed to drain under gravity for 72 h. Soil solutions were then extracted from these soils under vacuum, and 30 ml samples of these solutions were passed through 0.2 μm nitrocellulose membrane filters and acidified with 0.5 ml of ultrapure concentrated nitric acid. The dissolved concentrations of As, Pb and P were determined by inductively coupled emission-atomic emission spectrometry (ICP-AES) to be 3.19 ± 0.01, 0.56 ± 0.03 and 2.42 ± 0.01 mg L^{-1}, respectively.

The above-ground tissue of several plants of each cultivar was harvested in early July as a single composite sample by cutting all plants 2 cm above the soil surface, triple-washed with tap water and dried in paper bags at 70° C. This assay could not be replicated for each of the 16 cultivars collected because of the limited size of the plot, although duplicate samples of some of the more vigorous cultivars were taken to test for consistency in measured tissue concentrations of As and other trace elements in cultivars. However, the lack of replication means that the field assay cannot be used to reliably rank cultivars for their tendency to accumulate As, but is rather a survey of the range of tissue As that can be expected when growing arugula in As-contaminated orchard soils. Total fresh and dry weights varied greatly because of widely different cultivar growth rates and mature plant sizes. The dried tissues were ground, digested in nitric-perchloric acid on a hot plate and analyzed for As as well as Pb, Al, Fe and Ti by ICP-AES. Replicate samples and a certified plant standards (SRM Orchard Leaf 1571) were also analyzed to verify analytical accuracy and precision.

2.2. Hydroponic assay of arugula cultivars

The arugula cultivars were grown from seed hydroponically using complete Hoagland's nutrient solution at half-strength. This

Table 1
Concentrations of As, Pb, S and P in harvested tops of 16 arugula (*Eruca vesicaria subsp. sativa*) cultivars field-grown in an old orchard site with high soil As and Pb.

Cultivar	Accession ID	Origin	As (mg kg^{-1})	Pb (mg kg^{-1})	S (mg g^{-1})	P (mg g^{-1})
1	PI 650229	Sicily, Italy	177	43.2	8.59	3.86
2	Ames 7719	Minnesota, USA	134	8.5	9.61	4.43
3	PI 432339	Cyprus	106	14.0	9.06	4.03
4	PI 531331	Czechoslovakia	66.2	13.0	7.14	3.13
5	PI 597835	Algeria	134	25.9	6.71	4.28
6	PI 603033	Pakistan	128	11.0	7.82	3.67
7	PI 633205	Germany	70.5	11.6	7.80	3.73
8	PI 633209	Libya	145	19.9	7.43	2.93
9	PI 633216	Syria	89.2	16.9	6.19	3.52
10	PI 650174	Israel	110	17.9	8.72	3.24
11	PI 650177	India	173	11.4	7.97	3.86
12	PI 650183	Italy	129	23.9	5.29	3.09
13	PI 650185	Italy	158	17.2	4.77	2.90
14	PI 650186	Crete, Greece	98.4	15.5	6.74	3.67
15	PI 650209	Egypt	138	17.6	7.41	3.10
16	PI 650228	Sicily, Italy	133	14.2	6.92	3.21