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Influence of silicon on arsenic uptake and toxicity in lettuce

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Summary

Lettuce grown in soil is found to contain high concentrations of arsenic (As). This paper investigates the uptake and speciation of As in lettuce as well as the influence of silicon (Si) on As uptake, since Si may decrease it. Lettuce plants were cultivated in nutrient solution containing arsenite or arsenate with or without silicate. The uptake and distribution of As between roots and shoots, As accumulation in cell walls, As speciation, and toxic effects on growth were analysed. Results indicate that arsenite was more toxic to lettuce than was arsenate. Silicate decreased arsenate toxicity but had little effect on arsenite toxicity. In contrast, Si decreased arsenite uptake more than arsenate uptake. The concentration of arsenate was higher than that of arsenite in the plants independent of the As species added. When arsenate was added, the As concentration in shoots was half of that in the roots and this distribution did not change with Si addition. When arsenite was added, approximately 10 % of As was found in the shoots and 90 % in the roots; this pattern changed in the presence of Si, and As became evenly distributed in the plant. In both roots and shoots, approximately 40 % of the As was found in the cell wall fraction; when arsenite was added, the presence of Si increased this fraction to 47 %, but only in the shoots. The extraction efficiency when analysing the As species was lower in shoots than in roots, especially in the presence of arsenite and Si. The opposite was found for As concentration in pellets after extraction. This indicated variation in the binding strength of arsenite and arsenate between roots and shoots and between Si- and non-Si-treated plants.

Introduction

Arsenic (As) is ubiquitous in nature due to natural causes and anthropogenic activity. Arsenic is toxic to humans and may be taken into the human body via polluted waters and food (Bundschuh et al., 2012). Crop plants accumulate As and lettuce (*Lactuca sativa*) accumulates high As levels in the edible parts compared with other crops (Greger, 2006; Bergqvist et al., 2014).

Arsenic exists in various species in nature, the inorganic species arsenite and arsenate being predominant (Sadiq, 1997). In water or waterlogged systems, arsenite is the predominant As species, while arsenate is more common in terrestrial systems with higher redox potential (Sadiq, 1997). Small amounts of methylated arsenic species such as methylarsonic acid (MMA) and dimethylarsinic acid (DMA) may also be found due to microbiological activity (Wood, 1974). Plants mainly contain the inorganic As species arsenate and arsenite (Smith et al., 2008). Several plant species also contain methylated As species (Bergqvist and Greger, 2012). The levels of methylated As are usually low (Raab et al., 2007), and some argue that methylated As is not formed in plants but is taken up from the surroundings (Lomax et al., 2012). Arsenic is generally considered toxic to plants, such as lettuce (Sturchio et al., 2011). The toxicity, however, differs between As species. To most organisms, inorganic As species are regarded as more toxic than organic As species, and of the inorganic As species, arsenite is considered more toxic than arsenate (Meharg and Hartley-Whitaker, 2002).

Plants generally contain small amounts of As, especially in their aboveground parts, possibly due to restricted uptake and translocation from roots to shoots (Wang et al., 2002). However, some plants, such as the radish (*Raphanus sativus*), when cultivated hydroponically, accumulate higher concentrations of As in the shoots than the roots (Smith et al., 2008). Arsenite-sulphur compounds localized in the phloem could account for the high distribution to shoots in the radish (Smith et al., 2008). Much of the As in plants is bound in the apoplasmic cell-wall fraction, and 30-60 % was found in the apoplasm of rice (*Oryza sativa*) and Cretan brake (*Pteris cretica*) (Bravin et al., 2008; Feng et al., 2011). Cellular uptake of arsenate is thought to occur through the high-affinity phosphate transporters in terrestrial plants (Moreno-Jiménez et al., 2012), while cellular uptake of arsenite occurs through aquaporins used for glycerol and silicon (Bienert et al., 2008). The As accumulated in the cytoplasm is generally thought to be reduced to arsenite, bound to phytochelatins, and transported further into the vacuole (Moreno-Jiménez et al., 2012). Some plants additionally display arsenite cellular efflux ability when growing in an As-containing medium (Bienert et al., 2008).

Silicon is one of the most abundant elements on earth, but most of it has low availability to plants. Although Si is beneficial for some plant species, such as rice, bamboo, and sugar cane, its essentiality to other plants has been questioned (Epstein, 2009). Silicon was found to prevent the toxicity and decrease the uptake of toxic elements in plants (Treder and Cieslinski, 2005). Silicon may also alleviate the negative effects of As on plants; for example, in rice, Si inhibits the uptake of As, possibly due to the competition between silicic acid and arsenite (Bogdan and Schenk, 2008). Such effects, however, are not seen in the As hyperaccumulator Chinese brake (*Pteris vittata*), whose uptake mechanisms and routes differ from those of non-accumulators (Wang et al., 2010). Lettuce is not a Si-accumulator and not an As hyperaccumulator. The influence of Si on As uptake and toxicity to lettuce is therefore difficult to predict from data on Si-accumulators such as rice and As accumulators such as *Pteris vittata*.

Crops are cultivated on As rich soils, such as fertile alum shale soils, and lettuce is a popular vegetable to be cultivated. It is thus important to elucidate the influence of Si as an agent to decrease As uptake in lettuce. The aim of the study was therefore to investigate the effect of Si on the toxicity, accumulation, and speciation of As in lettuce (*Lactuca sativa*) when As was added as arsenate and arsenite. The hypothesis was that Si would decrease the arsenic uptake by, accumulation in, and toxicity to lettuce. The effect would likely be greatest when arsenite was added. Arsenite and silicate are thought to have the same uptake site on cell membranes; because Si decreases the cellular entrance of arsenite, a higher As level would be found in the apoplasm. More As would therefore accumulate in roots in relation to shoots with the addition of Si. By means of these effects, As toxicity will likely decrease with Si addition.

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Material and methods

Plant material

Seeds of lettuce (*Lactuca sativa* L. cv Amerikanischer brauner) were germinated in vermiculite for approximately 10 days until the plants were 1 cm tall. The plants were then transferred to 25%-strength nutrient medium; 100% medium contained the following concentrations of nutrients: 10 mM KNO_3, 1.5 mM KH_2PO_4, 3.0 mM $Ca(NO_3)_2$, 2.0 mM $MgSO_4$, 0.5 mM $(NH_4)_2 HPO_4$, 25 µM $FeCl_3$, 18.8 µM NaEDTA, 10 µM H_3BO_3, 16 µM $MnCl_2$, 0.16 µM $CuSO_4$, 0.35 µM $ZnSO_4$, and 0.21 µM Na_2MoO_4. The pH was 6.4. When the plants were 10 cm tall, they were transferred to 50%-strength nutrient medium, three plants per 1-L black pot. The nutrient solution was changed every week. Two weeks later, the plants were used in the toxicity tests and uptake experiments. Plants were grown in a climate chamber with a 16 h light/8 h dark regime at 23 °C/19 °C and a photon flux density of 270 µmol m^{-2} s^{-1} during the light period. The humidity was 80%.

Toxicity test

The plants were weighed and two plants were transferred to each 1-L pot containing 50%-strength nutrient solution. The solution was supplemented with various concentrations (i.e., 0, 0.1, 1, 10, 50, 100, 500, 1000, or 5000 µM) of arsenite ($NaAsO_2$) or arsenate ($Na_2HAsO_4 \times 7H_2O$), with or without 1 mM K_2SiO_3 added. The silicate concentration was chosen from a pilot study were 0-5 mM K_2SiO_3 and 0-10000 µM arsenate or arsenite was used under the same condition as described above. After five days treatment plants were divided into roots and shoots and weighed, dried at 80 °C for 48 hrs, and weighed again. The pH of the solution at the end of the five days of treatment was measured to be approximately 6.0 without and 6.5 with Si; four replicates were used.

Arsenic uptake

Two plants were transferred to each 1-L pot containing 50%-strength nutrient solution. After 24 h, the solution was replaced with a new nutrient medium containing 10 µM arsenite or arsenate in combination with 0 or 1 mM K_2SiO_3 added and treated during four days. The plants were then harvested and total As was measured in the whole tissue and in the cell wall fraction of the roots and shoots. In addition, the As speciation was analysed in the tissue fraction. This experiment was performed in four replicates.

Cell walls were prepared according to Lozano-Rodriguez et al. (1997) as follows. Plant material was first homogenized in liquid nitrogen using a mortar, and then ultra-mixed using a Polytron PT2000 homogenizer (Kinematica AG, Luzern, Switzerland) in extraction buffer containing 500 mM sucrose, 50 mM HEPES, 1 mM sodium dithionite, 5 mM ascorbic acid, and adjusted to pH 7.5. The sample was centrifuged at 500 g for 10 min. The pellet was used for As analysis of cell walls.

Short term arsenic uptake

Two times 1 ml samples were taken from 1-L pots containing 50%-strength nutrient solution in a combination of 1 µM arsenate or arsenite with or without 1 mM K_2SiO_3 added. One plant was placed in each1-L pot and 2 times 1 ml samples were taken after 10, 20, 30, 60 min, 2, 4, 6 and 24 hours. The total content of As in the samples was analysed in the samples using an atomic absorption spectrophotometer (SpectrAA 55B, Varian Inc., Palo Alto, CA) with the vapour generation technique (VGA-77, Varian Inc.). Sodium borohydride (3%; Merck, Whitehouse Station, NJ), sodium hydroxide (2.5%; EKA Chemicals), and hydrochloric acid (6M; VWR International, Radnor, PA) were used for hydride generation. Standards of As were added to the samples to eliminate matrix interaction effects. The detection limit was 7 µg As L^{-1}. The total As uptake was calculated based on plant fresh weight and the As content in the solution samples. Five replicates were used.

Analysis of arsenic

Cell walls and whole tissue of roots and shoots were dried at 80 °C for 48 hrs. The material was then wet digested in concentrated HNO_3:$HClO_4$, (7:3 v:v). The total content of As in the wet digested plant material, and solution from the short term uptake study, was analysed using an atomic absorption spectrophotometer.

Arsenic was extracted from plant material to analyse As speciation according to a modified Mir et al. (2007) method. Approximately 0.5 g DW of air-dried plant material was put in 50-mL Falcon tubes and then ultra-mixed using a Polytron PT2000 homogenizer (Kinematica AG) at maximum speed for approximately 10 s in 10 mL of MeOH:H_2O (1:1). The tubes were then placed in a sonicator (Transenic Digital S; ELMA, Singen, Germany) for 10 min followed by centrifugation at 3000 × g for 10 min. The supernatant was extracted into a new 50-mL Falcon tube. To each 50-mL Falcon tube, 10 mL of MeOH:H_2O (1:1) was added to the pellets for resuspension and the extraction procedure was repeated. In total, this extraction procedure was performed twice, rendering 20 mL of MeOH:H_2O (1:1) solution. The same procedure was then repeated two additional times with 0.1% HCl, rendering 20 mL of HCl solution. The volume of the extracted MeOH:H_2O (1:1) solution was reduced at 60 °C and resuspended in deionized H_2O to 3 mL to remove MeOH and to concentrate the low levels of organic As before analysis. In the case of HCl, the extracted solution was also concentrated to 3 mL.

Arsenic species in samples were separated using ion chromatography (IC) with a PRP X-100 (250 × 4.6 mm) anion exchange column (Hamilton, Reno, NV). Before injection of 100 µL of solution, samples were filtered through a 0.22-µm filter. The eluent was ammonium-phosphate buffer (pH 5.8) with a flow rate of 1 mL min^{-1}. The atomic absorption spectrophotometer (SpectAA 55B, Varian Inc.) vapour generation technique (VGA-77, Varian Inc.) was used for peak detection. Sodium borohydride (3%; Merck), sodium hydroxide (2.5%; EKA Chemicals), and hydrochloric acid (6M; VWR International) were used for hydride generation. Peak amounts of As were quantified by means of external calibration with standard solutions of arsenate, arsenite, MMA, and DMA using the following chemicals: sodium arsenate dibasic heptahydrate (Sigma-Aldrich, St. Louis, MO) for arsenate, sodium-*meta*-arsenite (Merck) for arsenite, sodium methylarsonate (Sigma-Aldrich) for MMA, and cacodylic acid (Sigma Aldrich) for DMA. SpectrAA Worksheet Oriented AA Software, Version 5.1 (Varian Inc.) was used to detect the peaks, and a formula devised by Burriel-Marti et al. (1968) was used to calculate peak area and implemented in Microsoft Office Excel.

Analysis of silicon

Roots and shoots of plants treated five days in nutrient medium added 0 and 1 mM K_2SiO_3 combined with 0 and 10 µM arsenite or arsenate, originating from the toxicity experiment, were dried at 80 °C for 48 hrs. The material was then wet digested in concentrated HNO_3:$HClO_4$, (7:3 v:v). The total content of Si in the wet digested plant material was analysed using atomic absorption spectrophotometty (Varian SpectrAA 55B) with furnace.

Calculations and statistics

The dose-response and sensitivity of various As and silicon combinations in the external medium were evaluated using the modified Weibull function (1) according to Taylor et al. (1991). This was

done for the increase in the fresh weight of the whole plants over five days.

$$y = a + b \exp[-(x/c)^d] \quad (1)$$

where y is the dependent variable, yield; x is the independent variable, the concentration of metal in the growth solution; a is the absolute minimum growth; b is the magnitude of the uninhibited growth response above the absolute minimum growth; and c and d indicate the shape of the curve, with parameter c altering the scaling on the x-axis and parameter d affecting the skewness of the dose-response (TAYLOR et al., 1991, 1992). The modified Weibull function provides direct estimates of some biological parameters of toxicity. These parameters are maximum unit toxicity (UT_{max}), defined as the maximum reduction in growth per unit of concentration of arsenic (2), and the empirical toxicity threshold (TT_{95b}), which is the concentration of arsenic that results in a yield reduction of 5% (3) (TAYLOR et al., 1991). When d was <1, the UT_{max} was calculated as dy/dx at TT_{95b} (TAYLOR et al., 1991):

$$UT_{max} = bd/c^d \exp[-(d-1)/d]\, c^{(d-1)}\, [(d-1)/d]^{[(d-1)/d]} \quad (2)$$

$$TT_{95b} = c[(-\ln 0.95)^{(1/d)}] \quad (3)$$

When comparing two values, a higher TT_{95b} and a lower UT_{max} indicate that a plant is less sensitive to arsenic than is a plant with a lower TT_{95b} and a higher UT_{max}. The concentration of As that induced a 50% reduction in growth increase was also calculated (i.e., EC_{50} values).

The Student t-test and ANOVA were used to detect differences between the treatments and linear regression was used to find significant differences between lines. Significance levels at $p \leq 0.05$ were calculated using the statistical program JMP (version 10.0, 2012; SAS Institute Inc., Cary, NC).

Results

Arsenic affected the growth of lettuce to different extents depending on the As species added. Arsenite produced a more severe effect and inhibited the plant-growth increase at lower concentrations than did arsenate (Fig. 1, Tab. 1). The lower TT_{95b} and EC_{50} and higher UT_{max} values for arsenite than for arsenate indicate this. Plants died when treated with concentrations higher than 50 µM arsenite but survived arsenate at concentrations as high as 1000 µM (not shown). The dry weight:fresh weight (DW:FW) ratio increased with increasing As addition, as fresh weight was more affected than was dry weight (Tab. 2). The shoot:root ratio (based on fresh weight) did not change with arsenate addition, but decreased slightly with high arsenite additions (Tab. 2).

Addition of Si decreased the effect of arsenate on growth more than that of arsenite, and the presence of Si increased TT_{95b} and EC_{50} but decreased UT_{max} (Tab. 1). The Si effect was especially

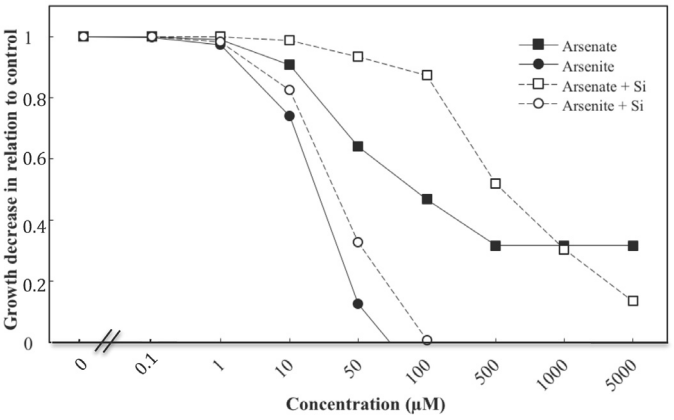

Fig. 1: Fresh weight decrease in relation to control of lettuce over five days of treatment with arsenite or arsenate in the presence or absence of 1 mM potassium silicate using modified Weibull function; ±SE is included in the figure, $n = 4$.

pronounced in the As concentration range of 10-100 µM (Fig. 1, Tab. 2). Although Si influenced the effect of As on fresh and dry weight, it did not influence the change in DW:FW ratio or shoot:root ratio caused by As (Tab. 2).

Lettuce accumulated more As in roots than in shoots (Tab. 3), independent of whether As was added as arsenite or arsenate. The $[As]_{shoot}$:$[As]_{root}$ ratio was lower when As was added as arsenite than as arsenate. When adding Si to the arsenite treatment, however, the As concentration increased in shoots and decreased in roots compared with the treatment without Si. No such Si effect was found in the arsenate case. The same concentrations found in plant tissue were also found in the cell wall fraction. Approximately 40% of As in the plant tissue was found in the cell walls in both roots and shoots. However, when Si was added to the arsenite treatment, a larger percentage, 47%, of As was bound to the cell walls in shoots.

The Si concentration in lettuce roots increased about 30% when arsenite or arsenate was added but not Si (Fig. 2). Similar effect was found in shoots, but only when arsenite was added. In the presence of 1 mM Si, however, the Si concentration in both roots and shoots decreased upon addition of arsenite or arsenate. This decrease was larger in shoots than in roots and As species added did not influence the magnitude.

The short-term uptake of As is shown in Fig. 3. In the first 30 min, when apoplasmic uptake occurs, treatment with arsenite + Si resulted in a lower uptake rate and the curve was saturated at a lower internal As concentration than in the other treatments. In the later part of the curve, showing symplasmic uptake, less As was taken up from the arsenite solution than the arsenate solution; furthermore, when Si was added, less As was taken up from either the arsenate or arsenite solution.

The effects of various treatments on As speciation in plant tissue are presented in Tab. 4. In all cases, arsenite and arsenate were found in

Tab. 1: Assessing response of fresh weight increase of lettuce to various combinations of arsenite or arsenate with potassium silicate using the modified Weibull function (see Calculations and statistics).

Treatment	Weibull parameter					TT_{95b}	EC_{50}	Ut_{max}
	a	b	c	d	R^2	(µM As g^{-1} FW)	(µM As g^{-1} FW)	(%)
Arsenate	0.505	1.095	67	1.01	0.455	3.53	46.5	0.016
Arsenate + Si	0.170	1.095	615	1.01	0.685	32.50	428.0	0.002
Arsenite	– 0.406	1.953	42	1.01	0.585	2.25	29.5	0.044
Arsenite + Si	– 0.368	1.743	66	1.01	0.684	3.47	45.8	0.025

Tab. 2: Dry weight (DW), dry weight:fresh weight ratio (DW:FW) of whole plants, and shoot:whole plant ratio based on fresh weight of lettuce after treatment for five days with various concentrations of arsenite or arsenate with or without 1 mM potassium silicate; $n = 4$, ±SE.

	Change in weight with As addition, %								DW:FW				Root:Shoot			
As, μM	Arsenate		Arsenate + Si		Arsenite		Arsenite + Si		Arsenate	Arsenate + Si	Arsenite	Arsenite + Si	Arsenate	Arsenate + Si	Arsenite	Arsenite + Si
	DW	FW	DW	FW	DW	FW	DW	FW								
0	100	100	100	100	100	100	100	100	5.58 ± 0.69	5.89 ± 0.46	5.58 ± 0.69	5.89 ± 0.46	82.9 ± 1.24	82.0 ± 1.01	82.9 ± 1.24	82.0 ± 1.01
0.1	93	80	104	90	95	76	99	91	5.15 ± 0.32	7.15 ± 0.82	5.98 ± 0.33	5.38 ± 0.12	79.9 ± 0.35	79.2 ± 1.70	79.1 ± 1.97	82.6 ± 0.20
1	95	83	98	82	94	75	108	118	6.26 ± 0.83	6.71 ± 0.83	5.82 ± 0.34	5.91 ± 0.06	77.9 ± 1.86	81.0 ± 1.35	82.5 ± 0.79	83.5 ± 0.86
10	88	54	103	107	98	77	96	57	6.26 ± 0.32	5.82 ± 0.73	6.57 ± 0.49	8.34 ± 0.16	77.9 ± 1.84	80.5 ± 1.27	82.2 ± 1.37	85.6 ± 0.96
50	90	57	105	91	88	43	91	42	6.77 ± 0.45	7.32 ± 0.24	8.11 ± 0.32	8.47 ± 0.58	82.9 ± 1.22	78.1 ± 0.92	81.9 ± 1.72	84.0 ± 0.29
100	92	58	98	62	81	24	91	45	6.85 ± 0.32	8.46 ± 0.56	8.40 ± 0.22	8.32 ± 1.38	78.9 ± 1.59	79.1 ± 1.27	81.9 ± 1.46	83.2 ± 1.31
500	91	51	103	80	85	26	82	20	7.74 ± 0.90	8.08 ± 0.47	11.11 ± 0.71	8.02 ± 0.40	83.4 ± 0.48	82.7 ± 1.86	78.7 ± 1.25	81.0 ± 1.13
1000	95	52	93	47	84	27	90	31	9.02 ± 1.18	8.33 ± 0.64	10.22 ± 0.49	10.88 ± 1.32	79.3 ± 2.41	79.9 ± 1.16	76.0 ± 0.76	77.8 ± 0.69
5000	95	59	89	38	79	17	80	12	8.15 ± 0.63	8.23 ± 0.02	9.59 ± 1.18	9.85 ± 2.46	82.1 ± 1.43	76.1 ± 2.39	73.5 ± 3.69	74.0 ± 2.98

both plant parts. Independent of the As species added, arsenate predominated in both roots and shoots. The arsenite:arsenate concentration ratio was higher when arsenite was added, and this ratio was higher in shoots than in roots. The addition of silicon decreased the arsenate and arsenite concentrations in both roots and shoots, either significantly or as a tendency. Adding silicon did not change the ratio between arsenite and arsenate in either roots or shoots.

Monomethylarsonic acid was detected in both the absence and presence of Si, but only when arsenate was added (Tab. 4). Silicon addition decreased the MMA concentration in both plant parts. To determine whether the MMA concentration in plants was due to bacterial activity in nutrient medium containing arsenate, sterilized and unsterilized media without plants were analysed after four days. The results indicated that the unsterilized solution contained 0.03 ± 0.006 (SE) μM MMA, while the sterilized solution contained no MMA (not shown). Arsenite or arsenate concentrations did not differ between the sterilized and unsterilized solutions and no DMA was detected. No DMA was found in the plants after the various treatments (Tab. 4).

The extraction efficiency for the As species analysis of lettuce tissue was higher in roots (≥60%) than in shoots (31-45%; Tab. 4). The addition of Si reduced the extraction efficiency in shoots but significantly only in the shoots of arsenite-treated plants, where the efficiency decreased from 45% to 13%. During extraction for As species analysis, a pellet fraction was formed as a by-product. When Si was added, a higher percentage of As was found in this pellet fraction for the shoots of arsenite-treated plants.

Discussion

This study demonstrated that silicon influences the toxicity, accumulation, and speciation of As in lettuce (*Lactuca sativa*).

As suggested by the hypothesis, the uptake of As, as both arsenite and arsenate, decreased when Si was present (Fig. 3). Arsenite, arsenate, and silicate may interact with each other at uptake into the root tissue. The interaction between arsenite and silicate was likely strongly antagonistic in the cell wall sites, since the apoplasmic uptake of As, as shown in the first 30 min in Fig. 3, was significantly affected by Si only when As was added as arsenite. The ionic interactions in the cell walls resulting in the decreased arsenite accumulation in the apoplasmic compartments could have been due to Si-induced secondary cell wall modifications (Yamamoto et al., 2012), for example, of As binding functional groups (Vithanage et al., 2012), reducing the binding affinity for arsenite. Low cell wall binding may increase the efflux of arsenite from roots, as suggested by Bienert et al. (2008). In addition, lack of binding in root apoplasm may increase As translocation to shoots, increasing the As concentration in the shoots and reducing it in roots (Tab. 3). At the same time As addition decrease the translocation of Si to the shoot; the decrease of Si concentration of shoots more than that of roots (Fig. 2), showing that interaction between Si and As occurs in both directions.

The effect of Si on the cellular uptake of As (shown after 30 min in Fig. 3) was, on the other hand, similar for arsenite and arsenate. Silicate might therefore interact with arsenite at aquaglyceroporins, i.e., the site of both arsenite and silicon uptake (Meharg and Jardine, 2003; Bienert et al., 2008), and with arsenate at the high-affinity phosphate transporters (Guo et al., 2007; Moreno-Jiménez et al., 2012).

After being taken up, As distribution in the plants depended on the As species added and on whether or not Si was present. Most As was found in roots, especially when added as arsenite (Tab. 3). It is known that arsenite is taken up by the root cells, where it is detoxified by being bound to phytochelatins, while arsenate may be translocated further to the shoots (Meharg and Hartley-Whitaker, 2002). For this reason, more As may be found in the shoots when

Tab. 3: Arsenic concentration in whole tissue and cell walls of roots and shoots of lettuce after treatment with 10 µM arsenite or arsenate with or without 1 mM potassium silicate for four days; $n = 4$, ±SE.

Treatment	As concentration, µg gDW^{-1}				As in cell wall fraction, %	
	Whole tissue		Cell wall			
	Root	Shoot	Root	Shoot	Root	Shoot
Arsenate	1260 ± 106[a]	508 ± 54[c]	1255 ± 53[s]	511 ± 37[u]	40.7 ± 6.04	35.6 ± 2.06
Arsenate + Si	1082 ± 220[a]	404 ± 28[c]	1089 ± 199[s]	456 ± 47[u]	37.0 ± 2.76	38.9 ± 1.45
Arsenite	1131 ± 250[a]	179 ± 38[d]	1017 ± 211[s]	196 ± 35[v]	34.4 ± 1.41	38.8 ± 3.87
Arsenite + Si	560 ± 51[b]	488 ± 43[c]	547 ± 66[t]	581 ± 32[u]	38.2 ± 2.36	47.2 ± 3.57

Different letters indicate significant differences between values in each column.

added as arsenate than as arsenite, which was the case in this study (Tab. 3).

Silicon affected the distribution of As between roots and shoots in lettuce when added as arsenite, but not as arsenate (Tab. 3). Much more As was translocated to the shoots in the presence than in the absence of Si, resulting in similar concentrations in shoots and roots in the case of arsenite (Tab. 3). Silicate interaction might have inhibited the cellular uptake via aquaporines of arsenite consigning arsenite to instead be translocated to the shoot. Silicate might have promoted the transformation of arsenite to arsenate in roots, which was thereafter translocated to shoots. However, there was no change in the arsenate concentration, nor in the arsenite concentration, in shoots in the presence of Si (Tab. 4). Adding Si did not result in any changes in the ratio between arsenite and arsenate in either roots or shoots compared with the non-silicon treatments. This suggests that enzymes responsible for arsenate/arsenite metabolism (Moreno-Jiménez et al., 2012) were unaffected by Si.

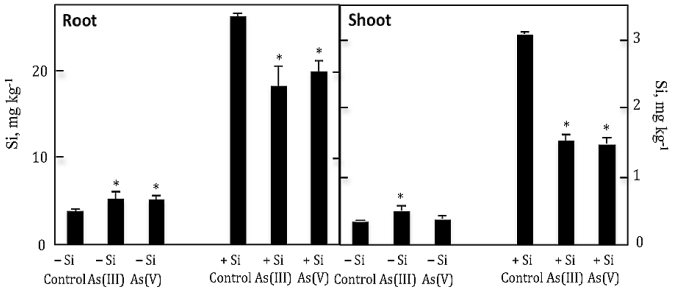

Fig. 2: Silicon concentration in root and shoot of lettuce after cultivation for five days in 0 and 1 mM potassium silicate with or without 10 µM arsenite (AsIII) or arsenate (AsV); $n = 9$, ±SE. * indicate significant difference from control.

The change in As concentration in shoots, but no change in arsenite or arsenate, might therefore depend on the increase of some other form of As in the shoots, for example, MMA or DMA. Dimethylarsinic acid (DMA) was, however, not found at all in lettuce (Tab. 4). Methylarsonic acid (MMA) was only found in roots and shoots when As was added as arsenate but not as arsenite, and the MMA concentration decreased in the presence of Si (Tab. 4). The MMA originated from the unsterilized nutrient solution (not shown) and was unlikely produced in the plant itself. Therefore, the formation of DMA or MMA could not contribute to the increased As concentration in shoots after Si addition.

One possibility of Si increases As concentration in shoot at arsenite addition, but no change in arsenite and arsenate, is that the addition of Si may decrease the ability of As to be extracted from the plant material. This is seen by the lower extraction efficiency in the arsenite + Si-treated lettuce (12%) than in the non-arsenite + Si-treated lettuce (44%; Tab. 4). Thus, As would be more bound to the shoot material in the presence than in the absence of Si. When analysing the different As species, the extraction efficiency was lower in shoots than in roots, meaning that the binding of As to the tissue likely differs between roots and shoots. Pellets remained from the extraction

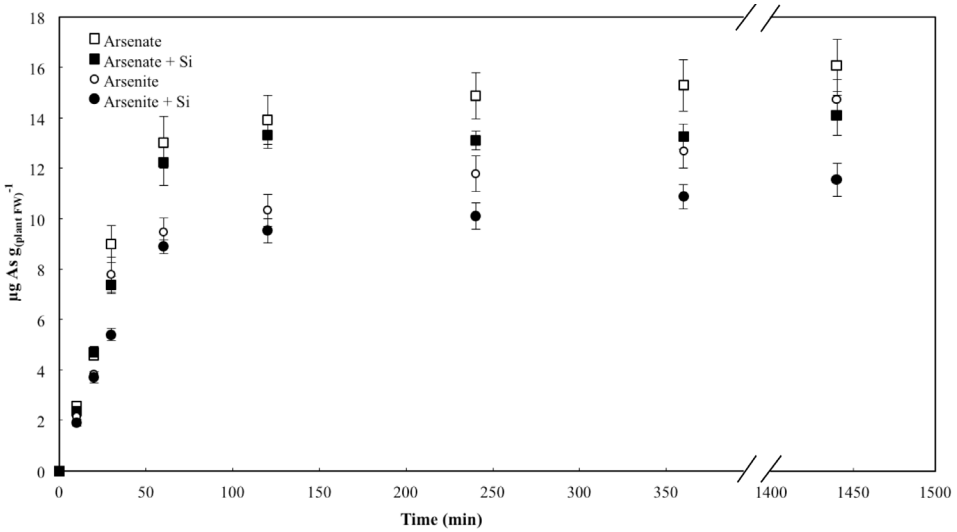

Fig. 3: Uptake of As by lettuce over 24 h from nutrient medium containing 1 µM arsenite or arsenate with or without 1 mM potassium silicate; $n = 5$, ±SE.

Tab. 4: Concentration of various As species (μg g^{-1} DW) in roots and shoots of lettuce after treatment with 10 μM arsenite or arsenate with or without 1 mM potassium silicate for four days. Indicated here is As in pellets left after extraction for As species in relation to total As in tissue as well as the extraction efficiency of arsenite species; $n = 4$, ±SE, nd = not detected.

Treatment		Arsenate	Arsenite	MMA	DMA	Extraction efficiency, %	As in pellet in relation to total As in tissue, %
Roots							
	Arsenate	1226 ± 50a	40.9 ± 1.34a	50.8 ± 2.38a	nd	58.0 ± 4.5a	6.9 ± 1.5ab
	Arsenate + Si	1109 ± 21a	29.9 ± 3.01b	43.7 ± 2.05a	nd	65.7 ± 10.4ab	22.1 ± 10.2abc
	Arsenite	1145 ± 74a	56.8 ± 0.43c	nd	nd	70.3 ± 19.5ab	8.0 ± 1.4ab
	Arsenite + Si	928 ± 61b	42.9 ± 4.98a	nd	nd	96.2 ± 6.7b	4.8 ± 1.7b
Shoots							
	Arsenate	180 ± 15a	43.0 ± 4.09a	49.7 ± 1.38a	nd	31.6 ± 4.6ab	45.6 ± 8.0c
	Arsenate + Si	92 ± 11b	21.3 ± 2.73b	27.9 ± 4.13b	nd	20.1 ± 2.9bc	31.4 ± 7.6c
	Arsenite	70 ± 3b	44.1 ± 0.28a	nd	nd	44.6 ± 10.4a	29.8 ± 10.7cb
	Arsenite + Si	61 ± 8b	40.0 ± 3.30a	nd	nd	12.6 ± 1.2c	83.9 ± 12.1d

Different letters indicate significant differences between values in the same column.

procedures before the analysis of arsenite and arsenate, and the As concentration in the pellets in relation to the total concentration in the tissue was higher in shoots than in roots (Tab. 4). In addition, in the shoots of arsenite-treated plants, the share of As in the pellet fraction in relation to the As in the tissue was even higher when Si was present (Tab. 4). The same trend was found for As in the cell wall fraction (Tab. 3). One suggested explanation of this is that the pellet material originated largely from the cell walls and that Si promoted a tighter binding of As to the cell walls, possibly by modifying functional groups in the walls responsible for As binding (Vithanage et al., 2012), thereby increasing the As concentration in the pellets.

In this study, we demonstrated that As was toxic to lettuce at elevated concentrations, and that arsenite was more toxic than arsenate (Fig. 1, Tab. 1 and 2). The latter has been demonstrated for other plant species as well (Meharg and Hartley-Whitaker, 2002). Less arsenite than arsenate was distributed to the shoots, likely to prevent the toxic effects of arsenite on the photosynthetic apparatus, which has also been seen in cucumber (*Cucumis sativus*; Uroic et al., 2012).

One would think from the uptake data, indicating that Si influenced the uptake of arsenite more than that of arsenate (Fig. 3), that Si would have influenced the toxicity of arsenite more than that of arsenate. However, the opposite was found. Silicate decreased the toxic effects of both arsenite and arsenate, but most efficiently decreased the toxicity caused by arsenate (Fig. 1, Tab. 1 and 2). One reason why Si generally decreased the arsenate and arsenite effect on growth was that Si decreased the net As uptake (Fig. 3), as also demonstrated by Bogdan and Schenk (2008). In addition, Si may increase antioxidant activities in plants, alleviating the negative effects of reactive oxygen species (Liu et al., 2009). Silicon would decrease the toxicity of arsenate more than that of arsenite because, when arsenate was added, both arsenite and arsenate concentrations decreased in both roots and shoots, while with arsenite addition, the concentration of both species decreased only in roots in the presence of Si (Tab. 3). In the latter case, therefore, the effect on photosynthesis would be the same as without added Si. In addition, Si might decrease the reactivity of the arsenate molecule.

We conclude that silicon can be used to decrease As uptake by lettuce, and thus, reducing toxic effects on the plant and, thus, in lettuce-crops. Lowered uptake also reduces the arsenic health-risk in lettuce as human food. During lettuce production, arsenate will be the major problem, while in solution culture, arsenite could be an additional problem due to the lower redox potential than in terrestrial conditions. In addition, cultivation in soil might include Si effects on the binding of As to soil colloids, which would in turn influence the As uptake (Seyfferth and Fendorf, 2012). Lettuce has a relative high uptake of arsenic compared to many other crop plants (McBride, 2013) but there are no international accepted limit for arsenic in food. There are suggestions eg. 200 mg/kg and 15 μg/kg b.w (EFSA, 2009). In field, lettuce can reach these levels and silicon would therefore be a useful tool to reduce As in food crops.

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