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REGULAR PAPER – PHYSIOLOGY/BIOCHEMISTRY/MOLECULAR AND CELLULAR BIOLOGY

Effect of phosphate on arsenic species uptake in plants under hydroponic conditions

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Abstract
Monothioarsenate (MTA) is a newly discovered arsenic (As) compound that can be formed under reduced sulfur conditions, mainly in paddy soil pore waters. It is structurally similar to arsenate As(V) and inorganic phosphate (Pi), which is taken up through phosphate transporters. Due to the similarity between As(V) and Pi, As(V) enters into plants instead of Pi. The important role played by phytochelatin (PC), glutathione (GSH), and the PC-vacuolar transporters ABCC1 and ABCC2 under As stress in plants is well known. However, the plant uptake and mechanisms surrounding MTA still have not been completely addressed. This investigation was divided in two stages: first, several hydroponic assays were set up to establish the sensibility-tolerance of wild-type *Arabidopsis thaliana* (accession Columbia-0, Col-0). Then Col-0 was used as a control plant to evaluate the effects of As(V) or MTA in (PC)-deficient mutant (*cad1–3*), glutathione biosynthesis mutant (*cad2*), and PC transport (*abcc1-2*). The inhibitory concentration (IC50) root length was calculated for both As species. According to the results, both arsenic species (As(V) and MTA) exhibited high toxicity for the genotypes evaluated. This could mean that these mechanisms play a constitutive role in MTA detoxification. Second, for the Pi-MTA and As(V)-Pi competition assays, a series of experiments on hydroponic seedlings of *A. thaliana* were carried out using Col-0 and a *pht1;1*. The plants were grown under increasing Pi concentrations (10 µM, 0.1 mM, or 1 mM) at 10 µM As(V) or 50 µM MTA. The total As concentration in the roots was significantly lower in plants exposed to MTA, there being less As content in the *pht1;1* mutant at the lowest Pi concentrations tested compared with the As(V)/Pi treatments. In addition, a higher rate of As translocation from the roots to the shoots under MTA was observed in comparison to the As(V)-treatments.

Keywords *Arabidopsis thaliana* · Arsenic uptake · Phosphate transporter · Phytotoxicity

Introduction

Arsenic (As), a metalloid element, can be mobilized naturally through biogeochemical cycles (Mitra and Paul 2020). It is found in abundance in the environment and is extremely harmful to all lifeforms (Singh et al. 2015). Because As is a class 1 carcinogen, the World Health Organization (WHO) has established a safe limit of 10 µg L^{-1} for As in drinking water (Niazi et al. 2018; Verma et al. 2020). This element occurs naturally in organic-rich black shales, Holocene alluvial deposits, volcanogenic sources, and thermal springs, as well as in anthropogenic activities such as mining, As-based fertilizer, fossil combustion, wood preservatives, and pesticides (Abedi and Mojiri 2020;Park et al. 2016; Rahman et al. 2014). Another important source to consider is the entry of arsenic into the human food chain, related to groundwater systems contaminated with this metalloid. These water sources are used for drinking, cooking, and irrigation of food crops (Shakoor et al. 2015). Arsenic exists in four oxidation states, (− 3), (0), (+3), and (+5) (Alka et al. 2020). Among these, As(V) is predominant in aerobic soils, while under reduced conditions like paddy soil, As is found in the form of arsenite (As(III)), which is more toxic than As(V) (Perez et al. 2021; Zhao et al. 2013).

Soil contaminated with heavy metal elements or metalloids is a major concern worldwide because of its ability

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to accumulate, persist, and move through food chains. For instance, plants growing in contaminated soils can take up the bioavailable metal fractions and accumulate them in their edible parts (Clemens 2019). This condition greatly depends on the nature of the contaminant and the type of plant, as well as the soil characteristics. In the case of As(V) (AsO_4^{3-}), which is analogous to phosphate (PO_4^{3-}), it can be incorporated into the metabolic pathways that require phosphate; this hampers discrimination between these ions in various transport processes (Ghosh et al. 2015; Niazi et al. 2017; Panda et al. 2010) and leads to the disruption of several cellular processes (Pickering et al. 2000). Nagarajan et al. (2011) and Remy et al. (2012) found that given the similarity between As(V) and Pi, in loss-of-function mutants of phosphate transporter proteins (PHT) in plants, less As(V) accumulates, leading to increased arsenic tolerance. However, PHT expression and its As(V) affinity can vary within the plants and between species. For instance, in *A. thaliana*, highly expressed *AtPht1;1* and *AtPht1;4* genes were observed in root tissues, which contributes to phosphate uptake at a low/high external concentration (Shin et al. 2004). In rice, large differences in the levels of various *OsPht* isoforms and isoform-specific responses to arsenic have been found (Wang et al. 2016). Most PHTs exhibit a slightly higher affinity for phosphate than for arsenic; nevertheless, PHT in *Pteris vittata* (identified as *PvPht1;3*) displayed a relatively high affinity for arsenic compared with other isoforms such as *PtPht1;5* (Ditusa et al. 2016). This may be linked to a hyper-accumulator-specific adaptation that contributes to the enhanced arsenic uptake and the accumulation observed in this species.

The As(V) toxicity mechanism mainly occurs via the replacement of Pi in key biochemical pathways, resulting in As(V) adducts that are short-lived and unstable. Thus, the formation and rapid autohydrolysis of As(V)-ADP establishes a futile cycle that uncouples oxidative phosphorylation and ATP synthesis. This reduces the ability of cells to produce ATP and carry out normal metabolism (Bhattacharya et al. 2012; Tawfik and Viola 2011). Furthermore, arsenic inhibits the photosynthesis rate in plants, since it reduces chlorophyll pigment synthesis and disturbs photosystem-I and -II activity by suppressing the key pathways (Gusman et al. 2013; Nagajyoti et al. 2010).

In recent years, the toxic activity of arsenic compounds that affects the cellular and metabolic pathways in plants has been studied intensively (Gautam et al. 2020; Gupta et al. 2020). After As(V) is taken up through PHT, it is reduced to As(III). This process is achieved by arsenate reductases that use glutathione (GSH) as a reductant (Farooq et al. 2016). In *A. thaliana*, the reductase high arsenic content 1 (HAC1) is expressed primarily in the root hairs, epidermal cells, and stele (Chao et al. 2014). Then As(III) is complexed by phytochelatins, followed by sequestration into vacuoles through ABCC vacuolar transporters (Zhao et al. 2013). However, As(V) and As(III) are not the only As species. For instance, thioarsenic is one of the principal arsenic species that has been discovered in high-arsenic groundwater, and its quantitative detection is critical for understanding arsenic transit in the environment (Shan et al. 2020). In addition, this arsenic species has been found in slags, geothermal fluids, wetland porewater, and flooded rice paddy soil (Herath et al. 2018). Because of this, interest in thioarsenical species has increased recently. The formation of thioarsenical species depends on As-transforming microorganisms, pH, S(–II)/As(III) and S(0)/As(III) ratios, and microoxic conditions in the soil, as well as on the presence of sulfate-reducing conditions (Bali and Sidhu 2021; Burton et al. 2013; Wang et al. 2020). Arsenic and sulfur are commonly found together in groundwater, and oxygen-bonded arsenic will be replaced by sulfur, generating As–SH and/or As=S substructures, which are known as thioarsenic compounds of thioarsenite and thioarsenate (Herath et al. 2018; Stucker et al. 2014). Monothioarsenate is the most stable of all the thioarsenates species (Planer-Friedrich et al. 2009). There are only a few studies in the literature that discuss the role of methylated oxoarsenicals and thioarsenical species in the accumulation of arsenic in plants. Therefore, the present investigation is aimed at deepening our understanding of MTA in plants. It is important to point out that both As(V) and MTA have tetrahedral structures. Due to the similarity between these two As-species, it is possible that MTA could stimulate effects similar to those observed in *A. thaliana* exposed to As(V). Because of this, As(V) was used for comparison in all of the experiments. The first part of this investigation was focused on the evaluation of the sensitivity-tolerance of MTA. The toxicity was tested by growing *A. thaliana* accession Columbia-0 (Col-0) in hydroponic cultures containing As(V) or MTA. In addition, three mutants in genes controlling the arsenic transport or metabolism associated with dealing with As(V) stress on the plant were also tested. The mutants evaluated were: (1) glutathione (GSH)-deficient mutant (*cad2*), which has a deletion of the gene encoding γ-glutamylcysteine (γ-EC) (Ha et al. 1999), (2) the phytochelatins (PC) synthase deficient mutant (*cad 1–3*), which is unable to synthesize PCs in response to cadmium (Cd) exposure (Ha et al. 1999; Howden et al. 1995a, b), and (3) the double-knockout mutant for the PC vacuolar transporters (*abcc1-2*) (Ha et al. 1999). These experiments were done in order to evaluate the effect of MTA on these mutant plants' lost function in certain genes important for As(V) plant responses.

Finally, the influence of Pi concentration on As(V) or MTA uptake in WT Col-0, as well as on *PHT1* loss-of-function plants, was tested in order to evaluate the MTA affinity for this transporter.

Materials and methods

As(V) or MTA toxicity treatment.

In order to evaluate the tolerance-sensitivity to As(V) or (MTA) in plants (in the hydroponic assays), a number of genotypes in *A. thaliana*, including wild type (WT), ecotype Columbia (Col-0), the AtPCS1 mutant for, *cad1-3*, the mutant *cad2*, and AtABCC1 and AtABCC2 double-knockout mutant (*abcc1-2*) were used. In the experiments, Columbia (0) (Col-0) was used as a control plant. The seeds were surface-sterilized by exposure to chlorine gas for 35 min, in accordance with Kühnlenz et al. (2014). The culture medium supporting plant growth was prepared based on a modified Hoagland solution no. 2, as described in Kühnlenz et al. (2014): 0.28 mM $Ca(NO_3)_2$, 0.6 mM KNO_3, 0.1 mM $(NH_4)H_2PO_4$, 0.2 mM $MgSO_4$, 4.63 µM H_3BO_3, 32 nM $CuSO_4$, 915 nM $MnCl_2$, 77 nM $ZnSO_4$, and 11 nM MoO_3. The Fe was supplied as *N*,*N*′-bis(2-hydroxybenzoyl) ethylenediamine-*N*,*N*′-diacetic acid (Fe- HBED) at a final concentration of 5 µM, in accordance with Chaney (1988). The medium was also supplied with 1% (w/v) sucrose and 0.05% (w/v) 2-(*N*-morpholino) ethanesulfonic acid (MES), and the pH was controlled at 5.7. Sucrose was introduced to stimulate seed germination in vitro; this carbohydrate provides energy to the plants, particularly when they are not yet ready to photosynthesize their food during the early stages of growth (Zahara et al. 2017).

Two different arsenate compounds, sodium arsenate dibasic heptahydrate ($Na_2HAsO_4{\cdot}7H_2O$; Fluka) and monothioarsenate (MTA) ($Na_3As^VO_3S{\cdot}7\ H_2O$), at a concentration range of 10–100 µM, were tested in order to investigate As toxicity. MTA (degree of purity 97%) was synthesized according to the method described by Schwedt and Rieckhoff (1996). The plates containing the medium with As species were sealed with Leucopore tape (Duchefa Biochemie, Haarlem, The Netherlands), followed by stratification at 4 °C for 2 days. For plant growth, the plates were then incubated for 9 days under a light/dark cycle of 16 h/8 h with a light intensity of 75 µeinsteins µE m^{-2} s^{-1} at 23 °C. After 9 days, the primary root length and the reduction ratio were determined. All experiments were performed in triplicate.

MTA uptake through phosphate transporters

The tolerance to As(V) or MTA was tested using WT, *A. thaliana* (L.) ecotype Columbia (0), and a mutant of phosphate transporter 1;1, *pht1;1*. The T-DNA insertion mutant (SALK_151938C) was obtained from the Nottingham Arabidopsis Stock Centre (NASC), Nottinghamshire, England. Before the arsenic and phosphate competition assays, it was verified that the mutant plants were homozygous. This condition was identified using LB primers (http://sig-nal.salk.edu/cgi-bin/tdnaexpress), gene-specific primers as designed by the SALK site iSect tool (http://sig-nal.salk.edu/tdnaprimers.html), Geneious 11.05 (https://www.geneious.com), and FastPCR, an in silico tool for fast primer, probe design, and advanced sequence analysis (http://primerdigital.com/fastpcr.html). The primer sequences used in this study are shown in Table 1, and the polymerase chain reaction (PCR) program is included in Table S1.

As(V) or MTA competition assays with phosphate

The plants (Col-0 and *pht1;1*) were grown hydroponically for 6 weeks. The description of the culture medium is in Table S2. After 2 days at 4 °C for stratification, cultivation started in agar-filled PCR tubes in pipet tip boxes for 4 weeks, followed by transfer into 50 mL tubes (Greiner Bio-One, Kremsmuenster, Austria) for another 2 weeks and exposure to 8 h of light at an intensity of 150 µE m^{-2} s^{-1} at 22 °C, followed by 16 h darkness at 17 °C. The medium was replaced with fresh medium twice a week in order to guarantee sufficient mineral and oxygen supply. After 6 weeks, the plants were transferred into 50 mL tubes containing a culture medium that was supplied with phosphate at a final concentration of 10 µM, 0.1 mM, or 1 mM, together with either As(V) or MTA at a final concentration of 20 µM and 50 µM, respectively (a total of six combinations). It should be pointed out that As(V) and MTA concentrations were selected on the basis of dose–response data. As(V) and MTA concentrations were measured on day 0, when the culture medium was without plant samples, and after 48 h. Note that the MTA was determined according to the procedure presented in Planer-Friedrich et al. (2007).

Table 1 List of oligonucleotides used as PCR primers for Genotyping in Wild type and mutant plants

Gene/ORF	Genotypes		Sequence
AT5G43350	Wild type	FW	5′ GCATGTCCAGACCTATTT CTCGC 3′
AT5G43350	Wild type	RV	5′ GAGGATGACGTCAAAGAC CCC 3′
AT5G43350	*pht1;1*	FW	5′ GCATGTCCAGACCTATTT CTCGC 3′
AT5G43350	*pht1;1*	RV	5′ TGGTTCACGTAGTGGGCC ATCG 3′

PCR program is available in supplementary information Table SI 1
FW forward primer, *RW* reverse primer

Total arsenic quantification in competition assays

Pooled root/leaf samples, including shoots, were separated, and the shoots were placed in paper bags. The roots were carefully washed with distilled water, followed by two washing steps, with 20 mM $CaCl_2$ and then with 10 mM ethylenediaminetetraacetic acid-di-Na (EDTA disodium salt), and a final washing step was performed with distilled water. Each washing step was performed with 20 mL per root sample at 4 °C for 10 min in order to remove any surface-bound As. The roots were then dried with a paper towel and placed in a paper bag. Thereafter, the roots and shoots were dried at 60 °C for 3 days.

Approximately 100 mg of the dried sample was used to determine the total As content (T-As). The sample was digested in 4 mL of 65% HNO_3 and 2 mL of 30% H_2O_2 in a microwave digestion system (START 1500 MLS GmbH with an HPR-1000/10 S high pressure segment rotor). Digested samples were analyzed for T-As via inductively-coupled plasma optical emission spectrometry ICP-OES (iCAP 6000 series, Thermo Scientific). The internal standard was the multi-element atomic spectroscopy standard solution V by Fluka Analytical.

Translocation factor

The ability of a plant to translocate the metals from the roots to the shoots is indicated by the translocation factor (TF), which can be represented by Eq. (1) (Malar et al. 2014; Radulescu et al. 2013):

$$TF = \frac{\text{Concentration of the heavy metal in the shoot}}{\text{Concentration of the heavy metal in the root}} \quad (1)$$

TF values less than 1 indicate that the plants are able to store heavy metals (HMs) in their roots, whereas values greater than 1 indicate greater translocation of HMs to the aerial parts (i.e., shoots and leaves) (Mellem et al. 2012).

Data analysis

Analysis of variance (ANOVA) was performed in order to evaluate the mean differences between parameters. Normality and variance homogeneity tests were performed using the Kolmogorov–Smirnov and Bartlett tests, respectively. A p- value ≤ 0.05 was used for all comparisons. A post hoc pairwise comparison by means of Tukey tests was used to confirm where the differences occurred between the groups against the control. When normality was not achieved, the Kruskal–Wallis test was used instead. All statistical analyses and graphical representations were carried out using GraphPad Prism software v. 6.01, San Diego, USA.

Results and discussion

As(V) or MTA toxicity in A. thaliana

The results of the hydroponic assay showed that root growth decreased as the concentration of the arsenic species in the medium increased. The comparison of the root growth values for Col-0 showed that As(V) was more toxic than MTA when the plants were subjected either to As(V) or MTA. For the mutants tested, *cad1-3* was the most sensitive, followed by *abcc1-2* and *cad2*. In general, the data clearly demonstrated a higher sensitivity of the mutant lines compared to that of Col-0 for both As(V) and MTA, with p value < 0.0001 (Fig. 1a, b). The half-maximal inhibitory concentration (IC_{50}) derived from the dose–response curve of the root length when the plants were exposed to As(V) was as follows: Col-0: 22.51 µM, *cad2*: 4.65 µM, *cad1*-3: 2.67 µM, and *abcc1-2*: 4.62 µM. The dose–response curve and the R^2 value for As(V)-IC_{50} calculation is shown in Fig. S1. The Col-0 exposed to a concentration of 100 µM As(V) led to a root length reduction of greater than or equal to 90%. A similar reduction in the root length of *cad2*, *cad1* − *3*, and *abcc1-2* were observed at As(V) concentrations ranging from 20 to 25 µM. The IC_{50} derived from the dose–response curve of the root length under MTA was as follows: Col-0: 31.99 µM, *cad2*: 10.69 µM, *cad1* − 3: 6.54 µM, and *abcc1-2*: 9.53 µM. The MTA-IC_{50} calculation curve is shown in Fig. S2. At 100 µM MTA, the root length of Col-0 was reduced by 62.9%. Regarding the mutant plants, the root length reduction was as follows: *cad2* 78.0% at 25 µM, and 90% at 50 µM MTA; *cad1*-3, and *abcc1-2* 85.6% and 74.6%, respectively, in the presence of 20 µM-MTA. A significant reduction in the root length of these genotypes was observed at concentrations above 25 µM-MTA.

Regarding the toxicity of As(V), the results of this study are in line with the report by Tang et al. (2016). They studied the tolerance of *A. thaliana* GSH-deficient mutant and PC-deficient mutant plants exposed to increasing As(V) concentrations on agar MS medium. As(V) conditions were 0, 50, or 100 µM for 20 days. They found that the root growth was completely inhibited at 50 µM As(V), as compared to Col-0, which had a root growth inhibition of only 20% under the same conditions. In another study, conducted by Planer-Friedrich et al. (2017), *A. thaliana cad1-3* was cultivated on agar plates. They found that the As(V)-/MTA-IC_{50} concentration ranged between 10 and 25 µM. From these findings, it can be concluded that the toxic effect of As on the plants grown in the liquid culture mediums was higher than that observed for the plants cultivated on agar plates.

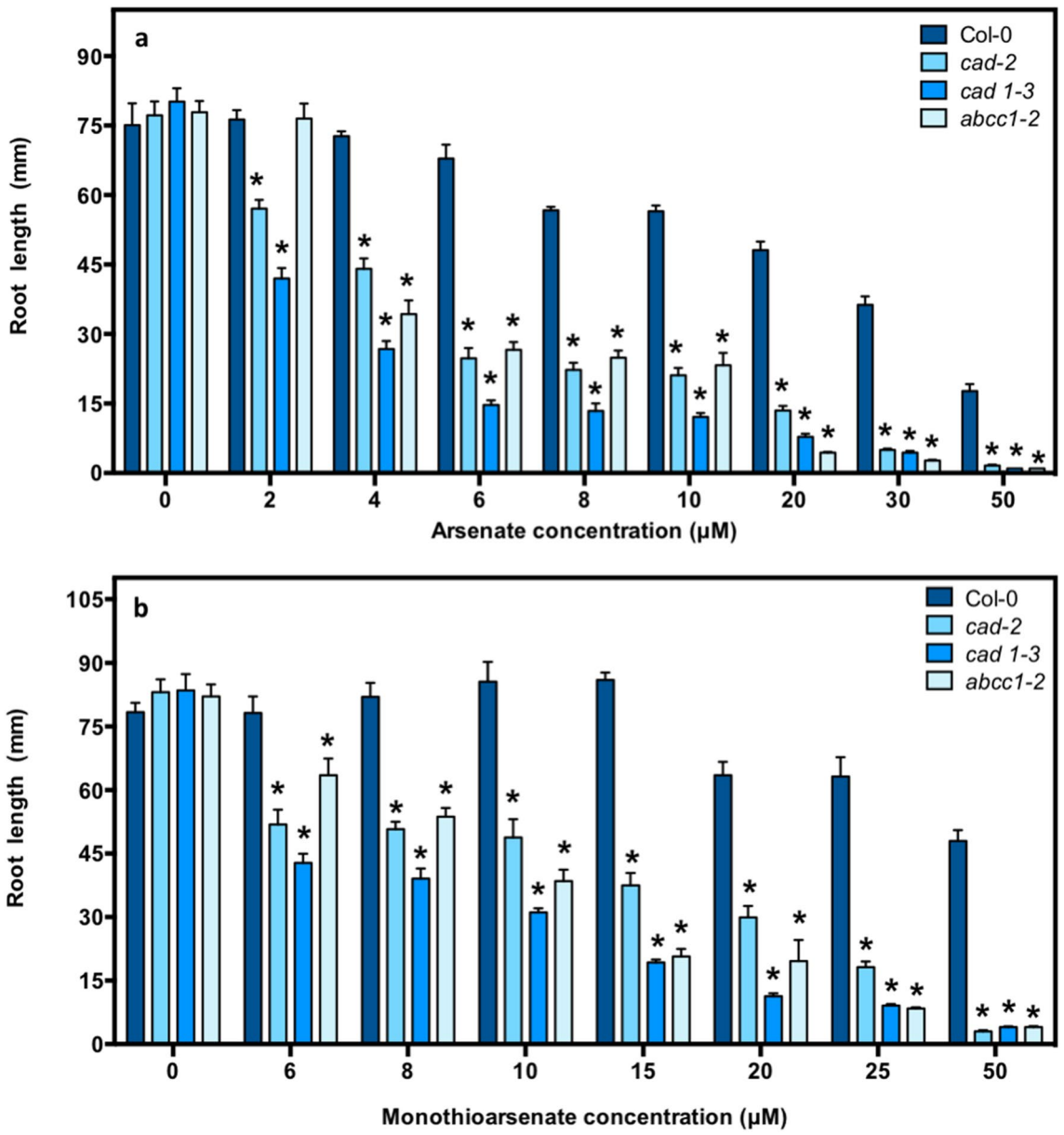

Fig. 1 Root lengths results of *A. thaliana* Col-0 in comparison with *cad2*; *cad1-3* and *abcc1-2*, in the presence of increasing concentrations of As(V) (**a**) and MTA (**b**) respectively. Asterisks above the bars indicate statistically different groups within the treatments. Significance is defined as $p \leq 0.05$. Data represent average ± SE

Aborode et al. (2016) assessed the sensitivity of young *A. thaliana* Col-0, *cad2*, and *cad1-3* to As(V) over a period of 10 days. They reported an average IC_{50} value of e 140 ± 14.2, 6.5 ± 0.4, and 6.0 ± 0.3 µM for the WT, *cad2*, and *cad1-3*, respectively. They highlighted the high toxicity observed in the mutants compared to the WT phenotype. The greater level of toxicity observed in this investigation in *abcc1-2* genotype was also described by Tang et al. (2016). They found that the root growth of *abcc1-2* was significantly suppressed by 5 µM As(V) and completely inhibited by 50 µM As(V).

According to a study by Song et al. (2010), the multidrug resistance-associated proteins (MRPs), a subfamily of adenosine triphosphate (ATP)-binding cassette transporters (more often abbreviated as ABC) in plants, play a key role in As detoxification. The high toxicity of MTA observed in this study on the *abcc1-2* mutant line could mean that this mechanism is probably activated under MTA too, according to the results. Studies in rice plants under 10 µM MTA for 72 h showed a significant reduction of MTA to As(III) in shoot and root tissues (Kerl et al. 2018). This reduction is also reported under As(V) in root cells. Then As(III) is

complexed by phytochelatins, followed by sequestration into vacuoles through ABCC vacuolar transporters (Zhao et al. 2013). The ABC transporters are implicated in heavy metal resistance by allowing the compartmentalization of a phytochelatins (PC)–As complex into the vacuole (Kamiya and Fujiwara 2011; Liu et al. 2010; Song et al. 2010).

Song et al. (2010) demonstrated that vacuoles isolated from *atabcc1 atabcc2* double knockout Arabidopsis plants exhibited only 10–15% residual As(III)-PC2 transport activity. This implies that *AtABCC1* and *AtABCC2* are the main PC transporters in Arabidopsis. Furthermore, the standalone overexpression of the transporters increased the plants' tolerance to As; an additional co-expression of PC synthase is required to attain the desired As-tolerant phenotype. Hence the sequestration of PC–As complexes into vacuoles plays an important role either in the detoxification of arsenic or in PC synthesis. The AtABCC1 and AtABCC2 transporters and PCS may function in a concerted way in the arsenic detoxification pathway (Song et al. 2010). This mechanism is also important in the plant's tolerance to Cd and Hg(II) (Park et al. 2012). The high sensitivity of the plants to As(V) and MTA indicated that the complexation of As(III) mainly depends on GSH, PCs, and subsequent sequestration in the vacuoles to protect cellular components from the reactive metalloid. These mechanisms, which play a constitutive role in the detoxification of inorganic As (Cobbett and Goldsbrough 2002; Liu et al. 2010; Zhao et al. 2009), could also be activated under MTA stress.

MTA uptake through phosphate transporter

As(V) or MTA toxicity in Col-0 and *pht1;1*

As(V) and MTA uptake was separately evaluated in Col-0 and *pht1;1* mutant for phosphate transporter 1;1 (PHT1;1). In *A. thaliana,* this gene is located on chromosome 5 (At5g43350), and it is involved in arsenate ion transmembrane transport, the cellular response to phosphate starvation, and phosphate ion transport (Phoenix Bioinformatics Corporation 2021). The *pht1;1* used in the experiments carries a T-DNA insert at the end of the second exon. The results of the genotyping of the homozygous line plants tested are shown in Fig. S3. In order to set an optimum concentration of MTA and As(V), the plants Col-0 and *pht1;1* were grown in the liquid medium for 9 days, and the dose–response curve was recorded. The As(V)-IC_{50} values for Col-0 and *pht1;1* were found to be 22.51 µM, and 22.40 µM, respectively. Although these IC_{50} values are close, it was noted that under 10, 20, 30, and 50 µM As(V) concentrations, the mutant line exhibited a subtle increase in the protective effect against arsenate. However, at higher concentrations of As(V) (100 µM), Col-0 activates mechanisms that allow them to deal with this stress condition, leading to values of root reduction length similar to that observed in *pht1;1* loss of function. This observation can be explained by the plants' ability to modulate their PTH transporters under As(V) stress. For instance, Navarro et al. (2021) found that *A. thaliana* can efficiently respond to As(V) variation levels under Pi starvation by modulating the transcription of PHT1;1. In the same way, it can control the expression of *PHT1*;1 through PHR1, a transcription factor that controls the *PHT1* expression under a Pi-starvation state (Bustos et al. 2010). In addition, Navarro et al. (2021) demonstrated that As(III) non-sequestered into the vacuole can regulate the expression of the *PHT1* through the modulation of the WRKY6 and ASK18 transcription factors in *A. thaliana*. A detailed explanation of the *PHT1* expression mechanism will be given in the section on the results of T-As arsenic accumulation in roots under As(V).

In the case of MTA, the IC_{50} values for Col-0 and *pht1;1* were 31.99 µM and 65.85 µM, respectively. The dose–response curve and the R^2 value of As(V)-IC_{50} and MTA-IC_{50} are shown in Figs. S4 and S5, respectively. The results showed a general decrease in the root length with an increase in As concentration from 0 to 100 µM for both As(V) and MTA. There was a significant difference between Col-0 and *pht1;1* at 5 µM As(V) and 15 µM MTA**,** Fig. 2a, b, respectively. At 100 µM As(V), the root length reduction for Col-0 and *pht1;1* was 88.9%, and 62.9%, respectively, while in the case of MTA, the root length reduction was 92.3% for Col-0 and 60.7% for *pht1;1*. Based on these findings, As(V) and MTA concentrations were set to 20 µM and 50 µM, respectively, to perform the competition assays in the presence of increasing phosphate concentrations, which were below the toxic threshold.

Total arsenic quantification in Col-0 and *pht1;1*

The hydroponic experiments were carried out using Col-0 and *pht1;1* mutant in the presence of As [either 20 µM As(V) or 50 µM MTA], where phosphate concentration increased from 10 µM to 0.1 mM and then to 1 mM. The results showed that T-As concentration in Col-0 and *pht1;1* decreased as the phosphate concentration in the growth medium increased. Similarly, the T-As concentration in the shoots under 10 µM Pi was 2.24 and 1.47 times higher for Col-0 and *pht1;1* genotypes, respectively, than that obtained at 0.1 mM and 1 mM Pi. The T-As concentration under 1 mM phosphate showed less variation compared to that at 10 µM and 0.1 mM Pi Fig. 3a. It was observed that Pi starvation resulted in an increase in the shoots' arsenic concentration. Similarly, the amount of T-As in the roots of Col-0 exposed to 20 µM As(V)-10 µM Pi was 3.4 times higher than that obtained in the presence of 0.1 mM Pi under the same As(V) conditions. By contrast, the lowest concentration of T-As was found at the highest concentration of

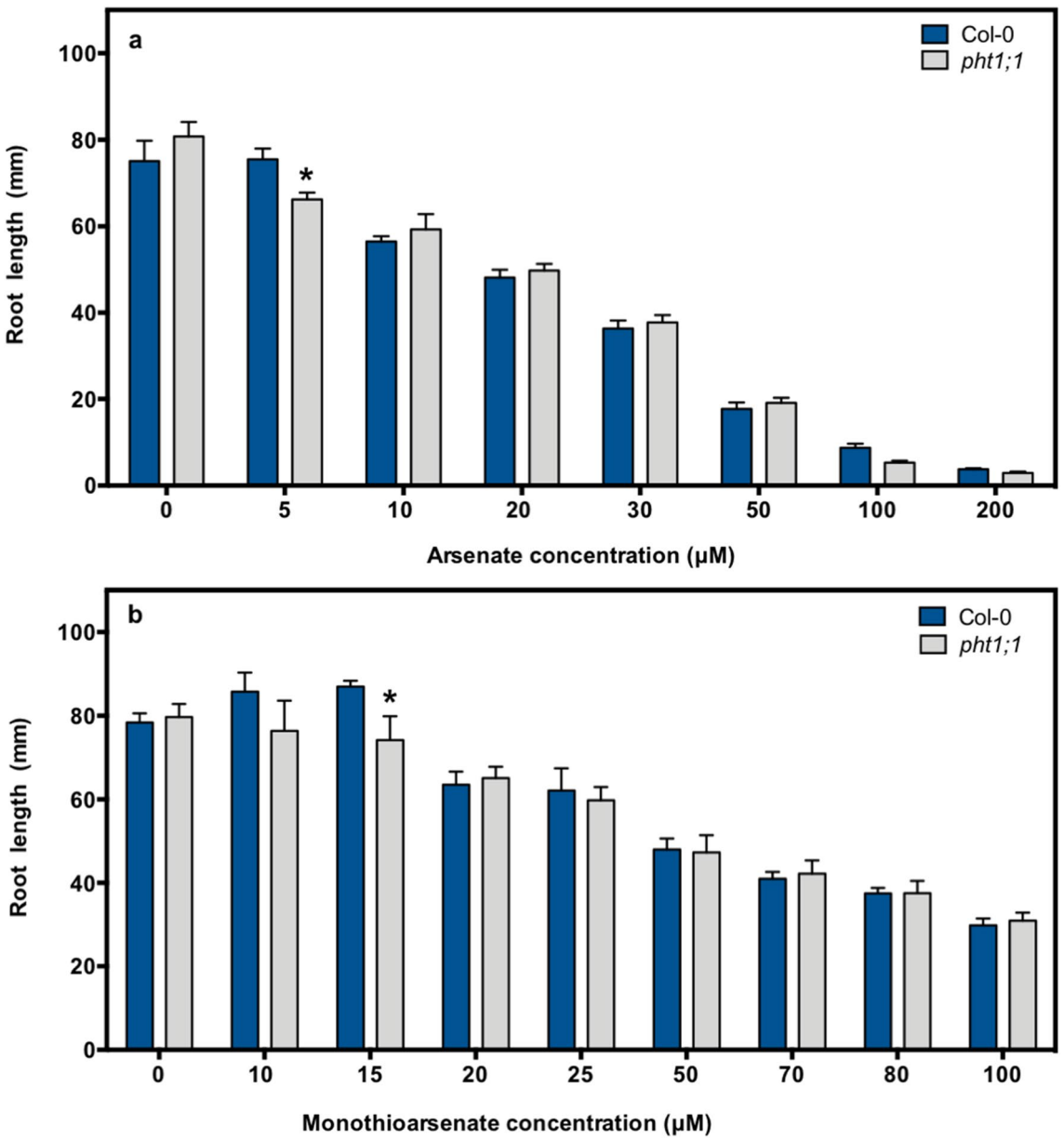

Fig. 2 Root lengths results of *A. thaliana* Col-0 in comparison with mutant for Phosphate transporter 1;1 PHT1;1, in the presence of increasing concentrations of As(V) (**a**) and MTA (**b**) respectively. Asterisks above the bars indicate statistically different groups within the treatments. Significance is defined as $p \leq 0.05$. Data represent average ± SE

phosphate (treatment 20 µM As(V)-1 mM Pi). For the roots of the *pht1;1*, the highest T-As was observed under 10 µM Pi, being 3.9 times higher than under 0.1 mM Pi. The lowest As concentration was found at 1 mM Pi under the same As conditions (Fig. 3b). The amounts of T-As in both shoots and roots for the plants tested under 20 µM As(V) and all Pi concentrations evaluated were not significantly different. This similarity in root arsenic concentration between Col-0 and *pht1;1* was also observed by Navarro et al. (2021), who found that in plants treated at 30 µM As(V), *PHT1;1* was repressed, and the As concentration found in the roots was similar to that of untreated plants. Similarly, the genetic expression of the Pi transporter involved in the ascension through the xylem (*PHO1*) was repressed as a response to the As(V) stress. They also noted that the regulation of certain transcription factors is closely related, because the *PHT1;1* recovery is dependent on the plant's capacity to detoxify As(V). This ability to control the phosphate transporters associated with reducing the stress caused by As(V) is related to the levels of arsenite As(III) in the external medium. Their results indicated that As(III) can regulate the As(V) uptake by influencing the action of WRKY6 (a

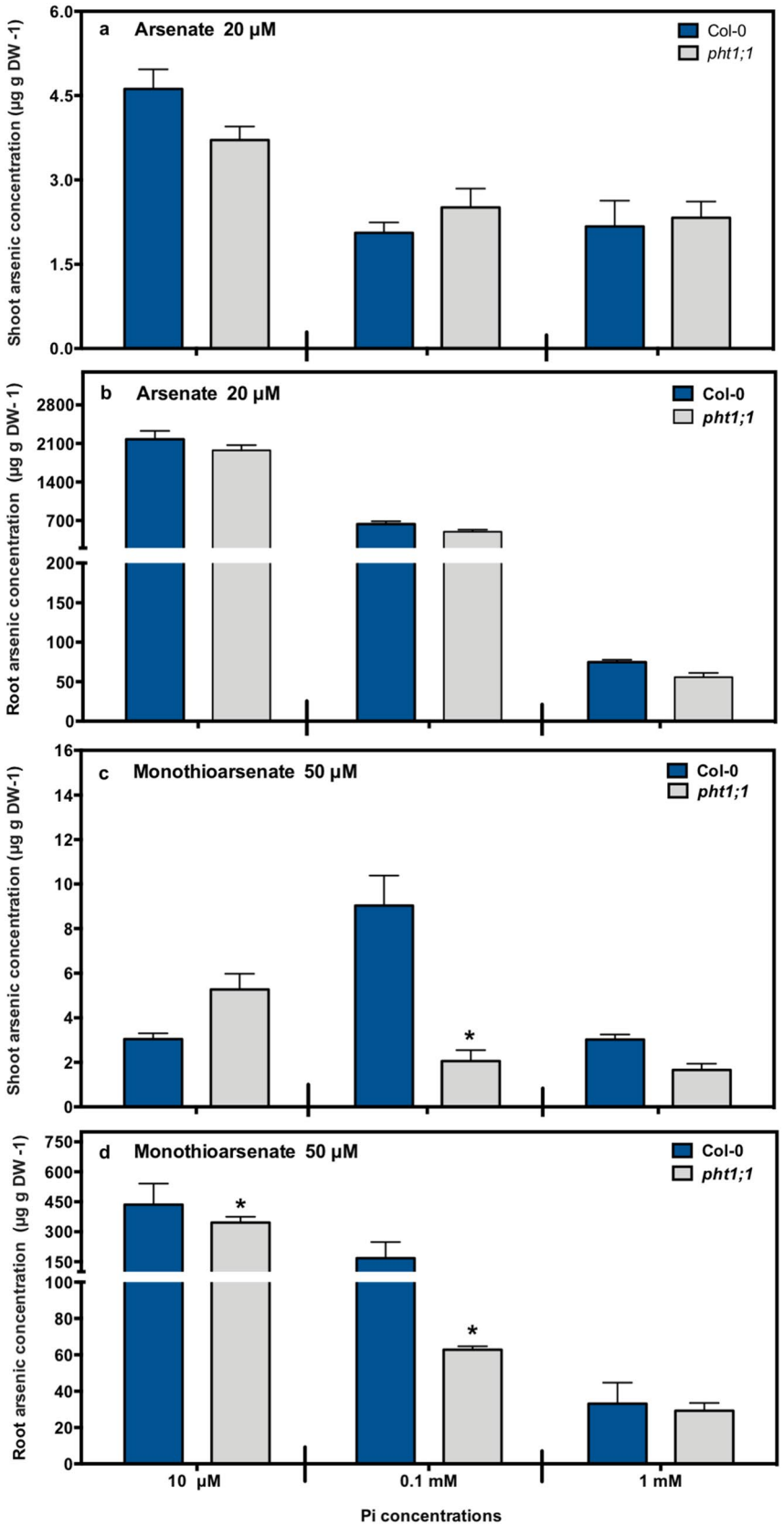

Fig. 3 Total arsenic accumulation in shoots and roots of Col-0 and *pht1;1* mutant growth in a hydroponic culture in the presence of increasing concentrations of phosphate: 10 µM Pi (insufficient condition), 0.1 mM Pi (sufficient condition) and 1 mM (more than sufficient condition) and under arsenic treatments 20 µM As(V) (**a**, **b**) or 50 µM MTA (**c**, **d**) respectively. Asterisks above the bars indicate statistically different groups within the treatments. Significance is defined as $p \leq 0.05$. Data represent average ± SE

transcription factor) on *PHT1;1*. Castrillo et al. (2013) found that under As(V) conditions, WRKY6 modulated the arsenate/phosphate transporter repression, which immediately restricts arsenate uptake. This repression was accompanied by delocalization of the phosphate transporter from the plasma membrane.

On the other hand, the role of other members of the PHT family should be emphasized. For instance, PHT1;4 is a protein of that group. In experiments carried out by Shin et al. (2004), they demonstrated that phosphate transporters PHT1;1 and PHT1;4 both have active participation in Pi uptake from the soil; moreover, both are implicated in arsenate transport. *pht1* null mutants are moderately arsenate tolerant, while *pht1* and *pht4* double mutants are significantly arsenate tolerant, indicating that arsenic uptake is compromised by mutations in these PHT proteins. The recent insights into the capacity of As(III) to regulate the expression of the *PHT1;1* gene as part of the response to the stress caused by As(V), as well as the contribution of PHT1;4 in As(V) uptake, are a possible explanation for the T-As results obtained in the present study under As(V) conditions. Furthermore, when plants overexpress *PHT1*, the sensitivity to As(V) increases (Catarecha et al. 2007). It is interesting to point out that under low concentrations of Pi, As(V) may outcompete Pi for uptake by plant roots. This could lead to symptoms of phosphorus deficiency (Finnegan and Chen 2012).

The highest amount of As in roots obtained in the present study was found at 10 µM Pi for both As(V) and MTA treatments. According to Azeem et al. (2017), Pi deficiency in nutrient solutions increased the capacity of the plants to take up more As(V), but high Pi treatment decreased As(V) uptake and toxicity. In *A. thaliana*, it has been shown that PHT proteins have an affinity for As(V) but not for As(III). Experiments carried out by LeBlanc et al. (2013) demonstrated that two Pi transporter family members, *AtPht1;1* and *AtPht1;7*, were hypersensitive to arsenate but unresponsive to As(III). The close link between As(V) and Pi was observed in other plants. For instance, in rice plants (*Oryza sativa*), Kamiya et al. (2013) evaluated the role of PHT transporters. In their experiments on *OsPht1;1*-deficient rice mutants, they found that the transcript expression level of *OsPT1* in *ospt1* was reduced by 70% in shoots and 50% in roots compared to that in the wild type, and arsenic concentrations in shoots were reduced by 60% compared to the wild type. Similarly, Wang and Duan (2009) grew *Oryza sativa* under 50 µM L^{-1} As(V) in a medium supplemented with 100 µM L^{-1} Pi. They observed that high Pi concentration decreased As uptake and high As concentration slightly decreased Pi uptake in the plants. Another example of the As(V)-Pi relation was seen in *Pteris vittata*, where the addition of 0.1–2 mM Pi showed that a high concentration of Pi resulted in a decrease in As(V) but not in As(III) accumulation in roots/shoots. The higher the Pi concentration, the more the reduction in As(V) uptake, whereas the higher the As(V) concentration, the greater the decrease in Pi uptake (Lou et al. 2010; Tu et al. 2004); Wang et al. 2002. In the case of *Lemna gibba*, the addition of 40 mg L^{-1} Pi reduced As(V) uptake (Mkandawire et al. 2004). Likewise, Sneller et al. (1999) examined As uptake in *Silene vulgaris* subjected to a Pi concentration in the range of 0.3–3 mg L^{-1}. They found that the root growth was not affected by As at high Pi concentration. However, at low Pi concentration, As had an adverse effect on root growth. The difference in the findings of the above-mentioned studies is because the authors used different types of soil and the hydroponic conditions were not the same.

Regarding the experiments at 50 µM MTA, the T-As amount in Col-0-shoots was about 3 times higher at 0.1 mM Pi than that at 10 µM Pi. By contrast, in *pht1;1*, the highest T-As amount (5.28 ± 2.09 µg g^{-1}) was found at 10 µM Pi and the lowest value was at 1 mM (Fig. 3c). The amount of As in the shoots of Col-0 and *pht1;1* mutant was observed to be different only at 0.1 mM Pi. In the case of the roots of the Col-0, the T-As amount at 10 µM Pi was 2.6 times higher than that at 0.1 mM Pi. The T-As amount at 1 mM was 5 times lower than that at 0.1 mM Pi. Similar to Col-0, in the root-*pht1;1* mutant line, a decrease in the As amount was observed when the Pi concentration increased in the medium. For instance, the amount of T-As at 10 µM was 5.5 times higher compared to that at 1 mM Pi (Fig. 3d). However, under the treatments at a constant concentration of 50 µM MTA, either at 10 µM Pi or at 0.1 mM Pi, significant differences were found between Col-0 and *pht1;1* ($p = 0.0001$). In general, the results of the competition assays clearly showed that phosphate influenced As uptake in the roots and shoots of the plants examined. This observation could be explained by the similarity in the chemical structure between Pi and As(V), and hence plants incorporated them into the metabolic pathways (Anawar et al. 2018; Meharg and Macnair 1992; Pickering et al. 2000). The results of this study are in good agreement with the findings of Pourrut et al. (2011). They noted that As uptake in plants decreased with an increase in phosphate concentration. The increase in the uptake of phosphate in the presence of arsenic has been reported as a tolerance mechanism by which plant cells can resist metalloid toxicity (Szegedi et al. 2010). The highest arsenic amount was observed under phosphate starvation (10 µM Pi); this indicated that the PHT1;1 showed an affinity for this thiolated As species. Under MTA conditions, *pht1;1* accumulated less arsenic compared to that of Col-0. However, the amount of As accumulated in the root cells under As(V) was higher than that observed in MTA treatments; this difference was seen regardless of Pi concentration over the range tested. The reason for this variation in the amount of arsenic in the roots exposed to As(V) or

MTA is not yet clear. The experiments conducted by Kerl et al. (2018) on rice exposed to MTA showed the same trend as in the T-As-roots results. The differences could be associated with the fact that the molecular weight of MTA is heavier than that of As(V), with values of 156 g mol^{-1} and 140 g mol^{-1}, respectively. In addition, the bond angles of MTA (91.85°) are smaller than those of phosphate and arsenate (109.5°) (Planer-Friedrich et al. 2017; Suess et al. 2009). In rice plants, a higher efflux was observed in roots and/or higher translocation to shoots in MTA-treated plants (Kerl et al. 2019).

In the results of the present study, for both As(V) and MTA treatments the influence of Pi on arsenic uptake was observed. According to Joardar (2014), it is clear that the addition of Pi to the soil alleviated As toxicity not only by decreasing As uptake by plants but also by increasing the plant biomass, Pi nutrition, and metabolic reactions. On the other hand, it must be understood that plants have developed different controlled adaptive mechanisms to acquire the necessary amount of external Pi and maintain Pi homeostasis, as well as to overcome low Pi availability (Rouached et al. 2010).

The effect of As(V) has been evaluated in other *A. thaliana* mutants lines. For instance, loss of function in *AtNIP:1* leads to reduced seed arsenic levels by affecting the long-distance transport of xylem and phloem (Lindsay and Maathuis 2016). Sun et al. (2016) demonstrated that in the *A. thaliana* mutant of inositol, pentakisphosphate 2-kinase (*AtIPK1*) decreased the As(V) tolerance. The As(V) uptake is lower in *atipk1-1* in comparison to the WT. On the other hand, Wang et al. (2017) evaluated the effect of As(V) in *A. thaliana* loss of function for HAC1 (an arsenate reductase), and phosphate effluxer (PHO1). At the same time, they tested the heterologous expression of the As-hyperaccumulator *Pteris vittata* arsenite efflux (PvACR3) in *A. thaliana*. They found that the combination of *PvACR3* expression with *HAC1* mutation led to As hyperaccumulation in the shoots. On the contrary, the combination of HAC1 and PHO1 mutation decreases As accumulation in *A. thaliana* shoots.

Concerning the TF results, the values showed a generally poor ability to translocate As from the roots to the shoots under both As conditions, As(V) or MTA, with an increase in Pi concentrations. This implies that As is mainly stored in the roots and remains in this tissue (Table 2). For the Col-0 and *pht1;1* plants, the highest TF value was found under 1 mM Pi in both As treatments. However, the highest TF values were found under MTA conditions. This observation is in good agreement with the results of Kerl et al. (2019), who determined the TF value in rice plants in the presence of either MTA or dimethylmonothioarsenate (DMMTA). The results of the present study demonstrated that MTA is taken up by plants, and a great amount remains in the root tissue and some is translocated to the shoots. In similar experiments on the European rice variety (*Oryza sativa* cv. Arelate) exposed to 10 µM MTA, Kerl et al. (2018) found that MTA is taken up through root cells and later is reduced to As(III). They also detected the presence of MTA in Xylem sap and root exudates. On the other hand, in this study it was observed that T-As uptake was lower upon exposure to MTA compared to that under As(V). However, effects such as the toxicity observed in plants exposed to MTA in a nutrient solution with low phosphate availability, as well as the accumulation of arsenic in plant roots exposed to MTA, make these observations, together with the reduction of the T-As concentration in *pht1;1*, possibly mean that this transporter has MTA affinity. All these facts could be indirect evidence that MTA uptake through phosphate transporters is similar to that of As(V). Thus, these results showed that MTA can contribute to arsenic stress in plants. Its potential contribution to grain As accumulation needs to be better understood.

Table 2 Translocation Factor (TF) for Col-0 and pht1;1 under different phosphate concentrations (Pi) and arsenic treatments 20 µM arsenate and 50 µM monothioarsenate

Genotypes	Pi	As(V) TF	MTA TF
Col-0	10 µM	0.002	0.007
Col-0	0.1 mM	0.003	0.054
Col-0	1 mM	0.029	0.091
pht1;1	10 µM	0.002	0.015
pht1;1	0.1 mM	0.005	0.033
pht1;1	1 mM	0.040	0.057

Translocation factor values < 1 indicated that metals were accumulated by the genotypes and largely stored in the roots, and translocation factor values > 1 indicated translocation of metals to the aerial parts of the plant

TF = translocation factor. This is dimensionless

Pi phosphate, *As(V)* arsenate, *MTA* monothioarsenate

Conclusions

The tolerance-sensibility evaluation showed that As(V) is more toxic than MTA in the genotypes tested. Higher toxicity was observed in *cad2*, *cad1 – 3*, and *abcc1-2*, in terms of a greater root-length reduction under both arsenic species [As(V) and MTA]. According to these results, in *A. thaliana*, GSH, PCs, and ABCC1 and ABCC2 played an important role in dealing with As(V) stress, and these responses were similar under MTA. This observation could indicate that these mechanisms are activated under this thioarsenical species in ways similar to those which plants use under As(V).

Regarding the PHT1;1-uptake experiments, under As(V), the amount of T-As accumulated in the roots of Col-0 and *pht1;1* were not significantly different over the whole Pi range (10 µM, 0.1 mM, and 1 mM Pi).

Nevertheless, under MTA conditions, significant differences were observed in the presence of the lower Pi concentrations in Col-0 compared to *pht1;1*. This demonstrates that the PHT1;1 transporter had some affinity for MTA. In general, the hydroponic experiments showed that the T-As concentration in the roots exposed to MTA-Pi was lower than that in the roots exposed to As(V)-Pi, which indicates more affinity to the PTH1 for As(V) than for MTA. In addition, when plants were exposed to MTA, compared to As(V), higher rates of As translocation from the roots to the shoots were found. The present study contributes to the elucidation of the possible uptake pathways for MTA, as well as confirming the role of GSH, PC, and ABCC1 and ABCC2 transporters as a part of the plants' response to MTA stress.

Further research needed

Despite a large amount of research devoted to deepening our understanding of the molecular mechanisms involved in arsenate uptake by plants, very little research has been reported on investigating the effect of MTA on plant growth. Therefore, the study of how plants respond when exposed to MTA should come into focus as a future research effort. In addition, the mechanisms of MTA translocation are not yet clear. It is also important to evaluate the affinity of other PHT family members for MTA and its contributions to the uptake process. Moreover, the transport system from the roots to the shoots should be assessed. Furthermore, a better understanding of MTA translocation will be especially important in order to prevent/reduce T-As accumulation in edible plant parts, especially in plants growing in flooded paddy soils, where the microbial transformation of inorganic arsenic to MTA is possible.

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References

Aborode FA, Raab A, Voigt M et al (2016) The importance of glutathione and phytochelatins on the selenite and arsenate detoxification in *Arabidopsis thaliana*. J Environ Sci (china) 49:150–161. https://doi.org/10.1016/j.jes.2016.08.009

Alka S, Shahir S, Ibrahim N et al (2020) The role of plant growth promoting bacteria on arsenic removal: a review of existing perspectives. Environ Technol Innov. https://doi.org/10.1016/j.eti.2020.100602

Anawar HM, Rengel Z, Damon P, Tibbett M (2018) Arsenic–phosphorus interactions in the soil–plant–microbe system: dynamics of uptake, suppression and toxicity to plants. Environ Pollut 233:1003–1012. https://doi.org/10.1016/j.envpol.2017.09.098

Azeem W, Ashraf M, Shahzad SM et al (2017) Phosphate–arsenate relations to affect arsenic concentration in plant tissues, growth, and antioxidant efficiency of sunflower (*Helianthus annuus* L.) under arsenic stress. Environ Sci Pollut Res 24:24376–24386. https://doi.org/10.1007/s11356-017-9977-2

Bali AS, Sidhu GPS (2021) Arsenic acquisition, toxicity and tolerance in plants—from physiology to remediation: a review. Chemosphere. https://doi.org/10.1016/j.chemosphere.2021.131050

Bhattacharya S, De Sarkar N, Banerjee P, Banerjee S, Mukherjee S, Chattopadhyay D, Mukhopadhyay A (2012) Effects of arsenic toxicity on germination, seedling growth and peroxidase activity in *Cicer arietinum*. Int J Agri Food Sci 2(4):131–137

Burton ED, Johnston SG, Planer-Friedrich B (2013) Coupling of arsenic mobility to sulfur transformations during microbial sulfate reduction in the presence and absence of humic acid. Chem Geol 343:12–24. https://doi.org/10.1016/j.chemgeo.2013.02.005

Bustos R, Castrillo G, Linhares F et al (2010) A central regulatory system largely controls transcriptional activation and repression responses to phosphate starvation in *Arabidopsis*. PLOS Genet 6:e1001102

Castrillo G, Sánchez-Bermejo E, de Lorenzo L et al (2013) WRKY6 transcription factor restricts arsenate uptake and transposon activation in *Arabidopsis*. Plant Cell 25:2944–2957. https://doi.org/10.1105/tpc.113.114009

Catarecha P, Segura MD, Franco-Zorrilla JM et al (2007) A mutant of the *Arabidopsis* phosphate transporter PHT1;1 displays enhanced arsenic accumulation. Plant Cell 19:1123–1133. https://doi.org/10.1105/tpc.106.041871

Chaney RL (1988) Plants can utilize iron form Fe-N,N′-di-(2-hydroxybenzoyl)-ethylenediamine-N,N′-diacetic acid, a ferric

chelate with 106 greater formation constant than Fe-EDDHA. J Plant Nutr 11:1033–1050. https://doi.org/10.1080/01904168809363867

Chao DY, Chen Y, Chen J et al (2014) Genome-wide association mapping identifies a new arsenate reductase enzyme critical for limiting arsenic accumulation in plants. PLoS Biol. https://doi.org/10.1371/journal.pbio.1002009

Clemens S (2019) Safer food through plant science: reducing toxic element accumulation in crops. J Exp Bot 70:5537–5557. https://doi.org/10.1093/jxb/erz366

Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182. https://doi.org/10.1146/annurev.arplant.53.100301.135154

Ditusa SF, Fontenot EB, Wallace RW et al (2016) A member of the Phosphate transporter 1 (Pht1) family from the arsenic-hyperaccumulating fern *Pteris vittata* is a high-affinity arsenate transporter. New Phytol 209:762–772. https://doi.org/10.1111/nph.13472

Farooq MA, Islam F, Ali B et al (2016) Arsenic toxicity in plants: cellular and molecular mechanisms of its transport and metabolism. Environ Exp Bot 132:42–52. https://doi.org/10.1016/j.envexpbot.2016.08.004

Finnegan PM, Chen W (2012) Arsenic toxicity: the effects on plant metabolism. Front Physiol 3:1–18. https://doi.org/10.3389/fphys.2012.00182

Gautam A, Kumar N, Dubey AK et al (2020) Sucrose plays key role in amelioration of arsenic induced phytotoxicity through modulating phosphate and silicon transporters, physiological and biochemical responses in C3 (*Oryza sativa* L.) and C4 (*Zea mays* L.). Environ Exp Bot. https://doi.org/10.1016/j.envexpbot.2019.103930

Ghosh P, Rathinasabapathi B, Ma LQ (2015) Phosphorus solubilization and plant growth enhancement by arsenic-resistant bacteria. Chemosphere 134:1–6. https://doi.org/10.1016/j.chemosphere.2015.03.048

Gupta K, Srivastava A, Srivastava S, Kumar A (2020) Phyto-genotoxicity of arsenic contaminated soil from Lakhimpur Kheri, India on *Vicia Faba* L. Chemosphere. https://doi.org/10.1016/j.chemosphere.2019.125063

Gusman GS, Oliveira JA, Farnese FS, Cambraia J (2013) Arsenate and arsenite: the toxic effects on photosynthesis and growth of lettuce plants. Acta Physiol Plant 35:1201–1209. https://doi.org/10.1007/s11738-012-1159-8

Ha S-B, Smith AP, Howden R et al (1999) Phytochelatin synthase genes from *Arabidopsis* and the yeast *Schizosaccharomyces pombe*. Plant Cell 11:1153 LP-1163 LP. https://doi.org/10.1105/tpc.11.6.1153

Herath I, Vithanage M, Seneweera S, Bundschuh J (2018) Thiolated arsenic in natural systems: what is current, what is new and what needs to be known. Environ Int 115:370–386. https://doi.org/10.1016/j.envint.2018.03.027

Howden R, Andersen CR, Goldsbrough PB, Cobbett CS (1995a) A cadmium-sensitive, glutathionedeficient mutant of Arabidopsis thaliana. Plant Physiol 107:1067–1073. https://doi.org/10.1104/pp.107.4.1067

Howden R, Goldsbrough PB, Andersen CR, Cobbett CS (1995b) Cadmium-sensitive, cad1 mutants of *Arabidopsis thaliana* are phytochelatin deficient. Plant Physiol 107:1059–1066. https://doi.org/10.1104/pp.107.4.1059

Joardar J (2014) Phosphate rich soil additive baked pig manure effectively reduces arsenic concentration in Japanese mustard spinach (*Brassica rapa* var. *perviridis*) grown with arsenic contaminated irrigation water. Am J Exp Agric 4:142–152. https://doi.org/10.9734/ajea/2014/6601

Kamiya T, Fujiwara T (2011) A novel allele of the *Arabidopsis* phytochelatin synthase 1 gene conferring high sensitivity to arsenic and antimony. Soil Sci Plant Nutr 57:272–278. https://doi.org/10.1080/00380768.2011.576398

Kamiya T, Islam R, Duan G et al (2013) Phosphate deficiency signaling pathway is a target of arsenate and phosphate transporter OsPT1 is involved in As accumulation in shoots of rice. Soil Sci Plant Nutr 59:580–590. https://doi.org/10.1080/00380768.2013.804390

Kerl CF, Rafferty C, Clemens S, Planer-Friedrich B (2018) Monothioarsenate uptake, transformation, and translocation in rice plants. Environ Sci Technol 52:9154–9161. https://doi.org/10.1021/acs.est.8b02202

Kerl CF, Schindele RA, Brüggenwirth L et al (2019) Methylated thioarsenates and monothioarsenate differ in uptake, transformation, and contribution to total arsenic translocation in rice plants. Environ Sci Technol 53:5787–5796. https://doi.org/10.1021/acs.est.9b00592

Kühnlenz T, Schmidt H, Uraguchi S, Clemens S (2014) *Arabidopsis thaliana* phytochelatin synthase 2 is constitutively active in vivo and can rescue the growth defect of the PCS1-deficient cad1-3 mutant on Cd-contaminated soil. J Exp Bot 65:4241–4253. https://doi.org/10.1093/jxb/eru195

LeBlanc MS, McKinney EC, Meagher RB, Smith AP (2013) Hijacking membrane transporters for arsenic phytoextraction. J Biotechnol 163:1–9. https://doi.org/10.1016/j.jbiotec.2012.10.013

Lindsay ER, Maathuis FJM (2016) *Arabidopsis thaliana* NIP7;1 is involved in tissue arsenic distribution and tolerance in response to arsenate. FEBS Lett 590:779–786. https://doi.org/10.1002/1873-3468.12103

Liu W-J, Wood BA, Raab A et al (2010) Complexation of arsenite with phytochelatins reduces arsenite efflux and translocation from roots to shoots in *Arabidopsis*. Plant Physiol 152:2211 LP-2221 LP

Lou LQ, Ye ZH, Lin AJ, Wong MH (2010) Interaction of arsenic and phosphate on their uptake and accumulation in Chinese brake fern. Int J Phytoremediat 12:487–502. https://doi.org/10.1080/15226510903051732

Malar S, Sahi SV, Favas PJC, Venkatachalam P (2014) Mercury heavy-metal-induced physiochemical changes and genotoxic alterations in water hyacinths [*Eichhornia crassipes* (Mart.)]. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-014-3576-2

Meharg AA, Macnair MR (1992) Suppression of the high affinity phosphate uptake system: a mechanism of arsenate tolerance in *Holcus lanatus* L. J Exp Bot 43:519–524. https://doi.org/10.1093/jxb/43.4.519

Mellem JJ, Baijnath H, Odhav B (2012) Bioaccumulation of Cr, Hg, As, Pb, Cu and Ni with the ability for hyperaccumulation by *Amaranthus dubius*. Afr J Agric Res 7:591–596. https://doi.org/10.5897/ajar11.1486

Mitra S, Paul D (2020) Iron plaque formation on roots and phosphate mediated alleviation of toxic effects in *Lens culinaris* Medik. induced by arsenic. S Afr J Bot 131:267–276. https://doi.org/10.1016/j.sajb.2020.02.024

Mkandawire M, Lyubun YV, Kosterin PV, Dudel EG (2004) Toxicity of arsenic species to *Lemna gibba* L. and the influence of phosphate on arsenic bioavailability. Environ Toxicol 19:26–34. https://doi.org/10.1002/tox.10148

Nagajyoti PC, Lee KD, Sreekanth TVM (2010) Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett 8:199–216. https://doi.org/10.1007/s10311-010-0297-8

Nagarajan VK, Jain A, Poling MD et al (2011) Arabidopsis Pht1;5 mobilizes phosphate between source and sink organs, and influences the interaction between phosphate homeostasis and ethylene signaling. Plant Physiol. https://doi.org/10.1104/pp.111.174805

Navarro C, Mateo-Elizalde C, Mohan TC et al (2021) Arsenite provides a selective signal that coordinates arsenate uptake and detoxification through the regulation of PHR1 stability in *Arabidopsis*. Mol Plant. https://doi.org/10.1016/j.molp.2021.05.020

Niazi NK, Bibi I, Fatimah A et al (2017) Phosphate-assisted phytoremediation of arsenic by *Brassica napus* and *Brassica juncea*: Morphological and physiological response. Int J Phytoremediat 19:670–678. https://doi.org/10.1080/15226514.2016.1278427

Niazi NK, Bibi I, Shahid M et al (2018) Arsenic removal by perilla leaf biochar in aqueous solutions and groundwater: an integrated spectroscopic and microscopic examination. Environ Pollut 232:31–41. https://doi.org/10.1016/j.envpol.2017.09.051

Panda SK, Upadhyay RK, Nath S (2010) Arsenic stress in plants. J Agron Crop Sci 196:161–174. https://doi.org/10.1111/j.1439-037X.2009.00407.x

Park J, Song W-Y, Ko D et al (2012) The phytochelatin transporters AtABCC1 and AtABCC2 mediate tolerance to cadmium and mercury. Plant J 69:278–288. https://doi.org/10.1111/j.1365-313X.2011.04789.x

Park JH, Han Y-S, Seong HJ et al (2016) Arsenic uptake and speciation in *Arabidopsis thaliana* under hydroponic conditions. Chemosphere 154:283–288. https://doi.org/10.1016/j.chemosphere.2016.03.126

Perez JPH, Schiefler AA, Rubio SN et al (2021) Arsenic removal from natural groundwater using 'green rust': solid phase stability and contaminant fate. J Hazard Mater 401:123327. https://doi.org/10.1016/j.jhazmat.2020.123327

Phoenix Bioinformatics Corporation (2021) The *Arabidopsis* information resource (Tair). In: Locus: AT5G43350. https://www.arabidopsis.org/servlets/TairObject?name=AT5G43350&type=locus. Accessed 15 June 2021

Pickering IJ, Prince RC, George MJ et al (2000) Reduction and coordination of arsenic in indian mustard. Plant Physiol 122:1171 LP-1178 LP. https://doi.org/10.1104/pp.122.4.1171

Planer-Friedrich B, London J, McCleskey RB et al (2007) Thioarsenates in geothermal waters of Yellowstone National Park: determination, preservation, and geochemical importance. Environ Sci Technol 41:5245–5251. https://doi.org/10.1021/es070273v

Planer-Friedrich B, Fisher JC, Hollibaugh JT et al (2009) Oxidative transformation of trithioarsenate along alkaline geothermal drainages—abiotic versus microbially mediated processes. Geomicrobiol J 26:339–350. https://doi.org/10.1080/01490450902755364

Planer-Friedrich B, Kühnlenz T, Halder D et al (2017) Thioarsenate toxicity and tolerance in the model system *Arabidopsis thaliana*. Environ Sci Technol 51:7187–7196. https://doi.org/10.1021/acs.est.6b06028

Pourrut B, Shahid M, Dumat C et al (2011) Lead uptake, toxicity, and detoxification in plants. Rev Environ Contam Toxicol 213:113–136. https://doi.org/10.1007/978-1-4419-9860-6_4

Radulescu C, Stihi C, Popescu IV et al (2013) Heavy metal accumulation and translocation in different parts of *Brassica oleracea* L. Rom J Phys 58:1337–1354

Rahman MA, Hogan B, Duncan E et al (2014) Toxicity of arsenic species to three freshwater organisms and biotransformation of inorganic arsenic by freshwater phytoplankton (*Chlorella* sp. CE-35). Ecotoxicol Environ Saf 106:126–135. https://doi.org/10.1016/j.ecoenv.2014.03.004

Remy E, Cabrito TR, Batista RA et al (2012) The Pht1;9 and Pht1;8 transporters mediate inorganic phosphate acquisition by the *Arabidopsis thaliana* root during phosphorus starvation. New Phytol 195:356–371. https://doi.org/10.1111/j.1469-8137.2012.04167.x

Rouached H, Arpat AB, Poirier Y (2010) Regulation of phosphate starvation responses in plants: signaling players and cross-talks. Mol Plant 3:288–299. https://doi.org/10.1093/mp/ssp120

Schwedt G, Rieckhoff M (1996) Separation of thio- and oxothioarsenates by capillary zone electrophoresis and ion chromatography. J Chromatogr A 736:341–350. https://doi.org/10.1016/0021-9673(95)01319-9

Shakoor MB, Niazi NK, Bibi I, Rahman MM, Naidu R, Dong Z, Shahid M, Arshad M (2015) Unraveling health risk and speciation of arsenic from groundwater in rural areas of Punjab, Pakistan. Int J Environ Res Public Health 12(10):12371–12390. https://doi.org/10.3390/ijerph121012371

Shan H, Liao D, Zhan H et al (2020) Development of LC-HGAFS method for direct measurement of monothioarsenate and application for its adsorption characteristics. Appl Geochem 122:104708. https://doi.org/10.1016/j.apgeochem.2020.104708

Shin H, Shin H-S, Dewbre GR, Harrison MJ (2004) Phosphate transport in *Arabidopsis*: Pht1;1 and Pht1;4 play a major role in phosphate acquisition from both low- and high-phosphate environments. Plant J 39:629–642. https://doi.org/10.1111/j.1365-313X.2004.02161.x

Singh R, Singh S, Parihar P et al (2015) Arsenic contamination, consequences and remediation techniques: a review. Ecotoxicol Environ Saf 112:247–270. https://doi.org/10.1016/j.ecoenv.2014.10.009

Sneller FEC, van Heerwaarden LM, Kraaijeveld-Smit FJL et al (1999) Toxicity of arsenate in *Silene vulgaris*, accumulation and degradation of arsenate-induced phytochelatins. New Phytol 144:223–232. https://doi.org/10.1046/j.1469-8137.1999.00512.x

Song W-Y, Park J, Mendoza-Cózatl DG et al (2010) Arsenic tolerance in *Arabidopsis* is mediated by two ABCC-type phytochelatin transporters. Proc Natl Acad Sci 107:21187 LP-21192 LP. https://doi.org/10.1073/pnas.1013964107

Stucker VK, Silverman DR, Williams KH et al (2014) Thioarsenic species associated with increased arsenic release during biostimulated subsurface sulfate reduction. Environ Sci Technol 48:13367–13375. https://doi.org/10.1021/es5035206

Suess E, Scheinost AC, Bostick BC et al (2009) Discrimination of thioarsenites and thioarsenates by X-ray absorption spectroscopy. Anal Chem 81:8318–8326. https://doi.org/10.1021/ac901094b

Sun Y-Y, Xu W-Z, Wu L et al (2016) An *Arabidopsis* mutant of inositol pentakisphosphate 2-kinase AtIPK1 displays reduced arsenate tolerance. Plant Cell Environ 39:416–426. https://doi.org/10.1111/pce.12623

Szegedi K, Vetterlein D, Jahn R (2010) Modelling rhizosphere transport in the presence of goethite, including competitive uptake of phosphate and arsenate. Plant Soil 330:481–501. https://doi.org/10.1007/s11104-009-0221-9

Tang Z, Kang Y, Wang P, Zhao FJ (2016) Phytotoxicity and detoxification mechanism differ among inorganic and methylated arsenic species in *Arabidopsis thaliana*. Plant Soil 401:243–257. https://doi.org/10.1007/s11104-015-2739-3

Tawfik DS, Viola RE (2011) Arsenate replacing phosphate: alternative life chemistries and ion promiscuity. Biochemistry 50:1128–1134. https://doi.org/10.1021/bi200002a

Tu S, Ma LQ, MacDonald GE, Bondada B (2004) Effects of arsenic species and phosphorus on arsenic absorption, arsenate reduction and thiol formation in excised parts of *Pteris vittata* L. Environ Exp Bot 51:121–131. https://doi.org/10.1016/j.envexpbot.2003.08.003

Verma G, Srivastava D, Narayan S et al (2020) Exogenous application of methyl jasmonate alleviates arsenic toxicity by modulating its uptake and translocation in rice (Oryza sativa L.). Ecotoxicol Environ Saf 201:110735. https://doi.org/10.1016/j.ecoenv.2020.110735

Wang L, Duan G (2009) Effect of external and internal phosphate status on arsenic toxicity and accumulation in rice seedlings. J Environ Sci (china) 21:346–351. https://doi.org/10.1016/s1001-0742(08)62275-5

Wang J, Zhao F-J, Meharg AA et al (2002) Mechanisms of arsenic hyperaccumulation in *Pteris vittata*. Uptake kinetics, interactions with phosphate, and arsenic speciation. Plant Physiol 130:1552–1561. https://doi.org/10.1104/pp.008185

Wang P, Zhang W, Mao C et al (2016) The role of OsPT8 in arsenate uptake and varietal difference in arsenate tolerance in rice. J Exp Bot 67:6051–6059. https://doi.org/10.1093/jxb/erw362

Wang C, Na G, Bermejo ES et al (2017) Dissecting the components controlling root-to-shoot arsenic translocation in *Arabidopsis thaliana*. New Phytol. https://doi.org/10.1111/nph.14761

Wang J, Kerl CF, Hu P et al (2020) Thiolated arsenic species observed in rice paddy pore waters. Nat Geosci 13:282–287. https://doi.org/10.1038/s41561-020-0533-1

Zahara M, Datta A, Boonkorkaew P, Mishra A (2017) The effects of different media, sucrose concentrations and natural additives on plantlet growth of Phalaenopsis hybrid "Pink." Braz Arch Biol Technol. https://doi.org/10.1590/1678-4324-2017160149

Zhao FJ, Ma JF, Meharg AA, McGrath SP (2009) Arsenic uptake and metabolism in plants. New Phytol 181:777–794. https://doi.org/10.1111/j.1469-8137.2008.02716.x

Zhao F-J, Harris E, Yan J et al (2013) Arsenic methylation in soils and its relationship with microbial arsM abundance and diversity, and As speciation in rice. Environ Sci Technol 47:7147–7154. https://doi.org/10.1021/es304977m

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