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REGULAR ARTICLE

Interactive effects of chromate and arsenate on their uptake and speciation in *Pteris ensiformis*

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Abstract
Background and aims Arsenate (AsV) and chromate (CrVI) inhibit each other's uptake and translocation in As-hyperaccumulator *Pteris vittata*. In the present study, we extended the research to As-sensitive plant *Pteris ensiformis* to better understand the mechanism of their interactions.
Methods Plants were exposed to 0, 0.75 or 7.5 mg L^{-1} AsV and 0, 0.52, or 5.2 mg L^{-1} CrVI for 7 d in hydroponics. Arsenic and Cr speciation were determined in nutrient solutions and plant biomass.
Results *P. ensiformis* accumulated high levels of As and Cr in the rhizomes and roots with low levels in the fronds. However, *P. ensiformis* was much more effective in taking up Cr than As, as much more Cr was accumulated in the roots (306–6015 vs. 87–642 mg kg^{-1}). AsV and CrVI increased each other's uptake in the rhizomes and roots when co-present. The AsV and CrVI taken up by *P. ensiformis* were reduced to arsenite (AsIII) and chromite (CrIII), possibly serving as detoxification mechanism.
Conclusions Uptake of As and Cr induced oxidative stress as indicated by increased lipid peroxidation and electrical conductivity. Arsenic and Cr increased each other's uptake by *P. ensiformis*.

Keywords Arsenic · Chromium · Speciation · Oxidative stress · Membrane damage · Electrolyte leakage test

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Introduction

Arsenic (As) and chromium (Cr) are common co-contaminants at hazardous waste sites, both are carcinogenic and of public health concern. Their concentrations in the environment have been increasing due to both natural and anthropogenic activities (de Oliveira et al. 2014, Shahid et al. 2017). High Cr concentrations in contaminated water at 30 mg L^{-1} in New Jersey, USA and 10 mg L^{-1} in Greece have been reported (Burke et al. 1991; Dermatas et al. 2017). Similarly, high As concentrations in groundwater has been reported worldwide. For example, groundwater contains up to 9 mg L^{-1} As in many regions of Bangladesh and 0.03–2.33 mg L^{-1} in Jianghan Plain, China (Ahsan and Del Valls, 2011; Gan et al. 2014). Since ingestion of contaminated crop food can be an important exposure route, it is important to understand the mechanisms of As and Cr uptake by plants.

Arsenate (AsV) and arsenite (AsIII) are two common forms of inorganic arsenic in the environment. AsV dominates in oxic environments and can strongly sorb to soil particulates, rendering it less mobile. AsIII is present under anoxic conditions and is more mobile, with AsIII showing greater toxicity to biota. The common valences of Cr in soils include chromite (CrIII) and chromate (CrVI), however, they differ in toxicity Módenes et al. 2017). While CrVI is soluble and toxic, CrIII is insoluble. Due to its strong oxidizing capability, CrVI is 100 times more toxic to biota than CrIII. Due to their toxicity, USEPA sets the maximum contaminant level (MCL) for As and Cr in drinking water at 10 and 100 μg L^{-1}. In 2014, the state of California mandated the MCL for CrVI at 10 μg L^{-1}. Due to their availability in soils, As and Cr can enter the food chain, presenting a potential health risk.

Plant As and Cr uptake by biota has been studied extensively. In general, plants take up AsV via phosphate transport systems, which can be rapidly reduced to AsIII in the roots and stored in different compartments, depending on plant species (Singh and Ma 2006; Ruíz-Torres et al. 2017). As a non-essential element, CrVI may be taken up by transporters for essential elements (Oliveira 2012) while plant CrIII uptake is a passive process with no energy being required (Shanker et al. 2005). As such, CrVI is likely taken up by plants through an active mechanism via sulfate, phosphate or iron transporters (Shanker et al., 2009; Kim et al., 2006; Kabir 2016), with energy expenditure and potential tissue damage resulting from the oxidative process. In most plants, As and Cr are mainly accumulated in the roots, with some being translocated to plant shoots.

As-hyperaccumulator *Pteris vittata* is efficient in taking up CrVI, which is reduced to CrIII and stored in the roots (Ma et al. 2001; de Oliveira et al. 2015). For example, *P. vittata* accumulated 686 and 39,800 mg kg^{-1} Cr in the fronds and roots after growing for 2 w in hydroponics containing 50 mg L^{-1} CrVI (Honda et al. 2015). Based on advanced X-ray spectroscopy, CrVI is reduced to CrIII in the roots. Additionally, Sridhar et al. (2011) reported that *P. vittata* was able to extract As and Cr from contaminated soil. Based on microscopic studies, Cr accumulation in *P. vittata* resulted in dehydration and cellular breakdown in the roots, which was not the case with As accumulation. This is consistent with de Oliveira et al. (2014) who reported 18% reduction in biomass after exposing *P. vittata* to 2.60 mg L^{-1} CrVI compared to 3.75 mg L^{-1} AsV for 7 d. Unlike *P. vittata*, the As-sensitive fern *P. ensiformis* has limited As accumulation potential (Singh and Ma 2006). After exposing *P. ensiformis* to 2 mg L^{-1} AsV for 14 d, it accumulated 17–71 mg kg^{-1} As in the biomass (He et al. 2017).

Plants respond to As and Cr stress by producing reactive oxygen species (ROS) in cells (Han et al. 2016). Arsenic enhances ROS production in both *P. vittata* and *P. ensiformis* (Srivastava et al. 2005; Singh et al. 2006). In addition, As and Cr induce oxidative stress in plants, leading to membrane lipid peroxidation and membrane damage (Singh et al. 2017). The impacts of AsV on the oxidative stress in *P. ensiformis* has been evaluated (Das et al. 2017), but no information is available on the impact of CrVI or the interaction between AsV and CrVI.

Studies on plant As and Cr uptake have focused on As-hyperaccumulators under high As and Cr exposure with few studies on their interactions in As-sensitive plants under low concentrations. As such, As-sensitive *P. ensiformis* was used for this study. The overall objective was to evaluate plant uptake and speciation in *P. ensiformis* under AsV and/or CrVI exposure. The specific objectives were to 1) investigate the effects of CrVI and AsV on each other's uptake and translocation in *P. ensiformis*, 2) determine As and Cr speciation in the media and plants, and 3) evaluate lipid peroxidation and membrane damage in *P. ensiformis* using the Evans Blue and electrolyte leakage tests. Better understanding of the mechanisms of As and Cr uptake and transformation in *P. ensiformis* may provide insight into As and Cr co-uptake in plants.

Materials and methods

Experimental setup

P. ensiformis plants of 8-month old with 5–6 fronds and ~12 cm in height cultivated in our lab were used. Efforts were made to ensure that the plants were uniform and similar in size. The plants were acclimatized in 0.2-strength Hoagland solution (0.2X HS) at pH 5.0 with 1 mM KOH-MES buffer for 4 weeks. They were then transferred to 1 L opaque containers with 0.2X HS containing different concentrations of arsenate (AsV) as $Na_2HAsO_4.7H_2O$ and chromate (CrVI) as $K_2Cr_2O_7$. Low concentrations were used to help understand the adaptive strategies of *P. ensiformis* under AsV and/or CrVI stress. Plants were exposed to 0, 0.75, or 7.5 mg L^{-1} AsV (0.01 or 0.1 mM AsV) and 0, 0.52, or 5.2 mg

L^{-1} CrVI (0.01 or 0.1 mM CrVI) for 7 d in hydroponic systems. Based on Visual MINTEQ 3 (Gustafsson 2011), the As and Cr in 0.2X HS were soluble and therefore available for plant uptake. The treatments are referred to $As_{0.75}$, $As_{7.5}$, $Cr_{0.52}$, $Cr_{5.2}$, $As_{0.75} + Cr_{0.52}$ and $As_{7.5} + Cr_{5.2}$.

The solutions were aerated continuously and renewed twice weekly during the experiment. All plants were grown in a greenhouse under a light photoperiod of 8 h, a light density of 350 μmol m^{-2} s^{-1}, mean temperature 25 °C and relative humidity of 70% (de Oliveira et al. 2014). There were 3 replicates for each treatment. Plants were harvested after 7 d of growth and were separated into the fronds, rhizomes and roots.

As and Cr concentrations and speciation in plants and growth media

After growing for 7 d under different As and Cr treatments, *P. ensiformis* was rinsed with deionized water and separated into above ground (fronds) and below ground (rhizomes and roots) biomass. To remove adsorbed As and Cr on surface, *P. ensiformis* roots were placed under running distilled water, rinsed with ice-cold phosphate buffer and washed once again with distilled water. Oven-dried (65 °C for 2 d) samples were digested with HNO_3/H_2O_2 for total As and Cr analysis using USEPA Method 3050B on a hot block (Environmental Express, Ventura, CA). The As and Cr concentrations in digested solutions were analyzed by inductively coupled plasma mass spectrometry (ICP-MS; Perkin-Elmer Corp., Norwalk, CT).

To monitor the species changes in Cr and As, the spent media of 0.2× HS were collected after 0, 1, 2, 3, 5 and 7 d of growing *P. ensiformis*. To minimize changes in AsV and CrVI, 5 mL of 0.2× HS was sampled and analyzed immediately. Specifically, concentrations of CrVI in 0.2X HS were measured using a colorimetric reagent specific for CrVI, 1,5-diphenylcarbazide, which was dissolved in 0.05% acetone (de Oliveira et al. 2015). Absorbance was measured with a spectrophotometer at 540 nm (UVI1800U, Shimadzu Corp., Columbia, MD).

Arsenic speciation in *P. ensiformis* plant was performed by extracting fresh plant samples ultrasonically in 10 ml of ethanol:water (1:1 *v*/v) twice for 4 h at 60 °C (Zhao et al., 2015). The extracts were decanted into a 50-ml volumetric flask and diluted to 50 ml with deionized water. Arsenate and AsIII were separated using an arsenic speciation cartridge (Waters SPE cartridge), which retains AsV (Mathews et al. 2011). Arsenic was determined by ICP-MS. In addition, standard reference material 1547 (peach leaves) from the National Institute of Science and Technology (Gaithersburg, MD) and appropriate reagent blanks, internal standards and spikes were used to ensure method accuracy and precision.

Hexavalent chromium concentrations in *P. ensiformis* plants were measured by ICP-MS using a modified method of alkaline digestion based on USEPA Method 3060A (US Environmental Protection Agency, 1995). Briefly, ~0.20 g of dry biomass was transferred into a 100 ml glass beaker with 20 ml of 0.1 M Na_2CO_3 and boiled on a hot-plate for 10 min (Khakhathi et al. 2011). After cooling, samples were filtered through Whatman no.1 filter paper and diluted to a volume of 50 ml with deionized water. During the extraction, CrVI was solubilized by Na_2CO_3 solution to form Na_2CrO_4 while CrIII species formed insoluble hydroxides or carbonates (de Oliveira et al. 2015).

Lipid peroxidation and membrane damage in plants

Thiobarbituric acid reactive substances (TBARS) have been used to estimate lipid peroxidation and membrane damage under metal exposure (Singh et al., 2015). Specifically, frozen *P. ensiformis* roots of ~ 0.3 g were cut into small pieces and homogenized using a cold mortar and pestle in an ice bath with 1.5 ml of 5% (wt/v) trichloroacetic acid (TCA) solution (de Oliveira et al. 2015). The homogenate was transferred into fresh tubes and centrifuged at 10,000 g for 10 min at room temperature. To 1 ml of the supernatant, 1 ml of 20% (*w*/*v*) TCA containing 0.5% (*w*/*v*) thiobarbituric acid was added. The mixture was heated at 95 °C for 30 min and then cooled quickly on ice. The absorbance of the supernatant was measured at 532 and 600 nm (Shimadzu UVI60U, Columbia, USA). The TBARS content was calculated using an extinction coefficient of 155 μmol g^{-1} fw (fresh weight) based on fresh biomass.

The impact of As and/or Cr on membrane stability of plant roots was evaluated using Evans Blue dye uptake test (Yamamoto et al. 2001). The stain cannot penetrate through the membrane of living cells, serving as an indicator of the loss in root plasma membrane integrity. The roots were stained with 0.025% (w/v) in Evans Blue solution in 100 mM $CaCl_2$ at pH 5.6 for 10 min. They

were then washed in 100 mM $CaCl_2$, homogenized with 1% (w/v) sodium dodecyl sulfate and centrifuged at 13,500 g for 10 min. The absorbance of the supernatant was measured at 600 nm.

In addition, electrolyte leakage has been used to estimate membrane integrity under stress (Rolny et al. 2011). Root membrane integrity based on membrane injury index was determined by recording the electrical conductivity after placing plant roots in deionized water at 40 °C and 100 °C for 15–30 min (Almeselmani et al. 2006). Root samples of 0.1 g were cut into uniform size and placed in test tubes containing 10 ml of deionized. One set was kept at 40 °C for 30 min and another set at 100 °C in boiling water bath for 15 min. The electric conductivities C40 and C100 were measured by a conductivity meter and injury index was calculated as C40 /C100× 100.

Statistical analysis

All data are expressed as means of three or four replicates with standard error. Analyses of variance (ANOVA) by Tukey's multiple grouping were used to determine significance of the interactions among treatments. All statistical analyses were performed with SAS statistical software (version 9.1.3, NC, USA).

Results and discussion

The plants were exposed to 0, 0.75, or 7.5 mg L^{-1} AsV and 0, 0.52, or 5.2 mg L^{-1} CrVI, which are referred to $As_{0.75}$, $As_{7.5}$, $Cr_{0.52}$, $Cr_{5.2}$, $As_{0.75}$ + $Cr_{0.52}$ and $As_{7.5}$ + $Cr_{5.2}$. We evaluated As and Cr concentrations and speciation in plants and growth media, determined plant oxidative stress based on TBARS, and evaluated root membrane damage and stability based on Evans Blue dye uptake test and membrane injury index. The plants were cultivated hydroponically to better understand the interaction and speciation of As and Cr in the plant and media (Adrover et al. 2013).

Exposure to AsV and CrVI caused root damage in *P. ensiformis*

Both As and Cr are non-essential toxic elements, which interfere with metabolic and physiological processes in plants (Kabata Pendias 2010). Exposure to As and/or Cr often reduces biomass in plants. For example, Cr toxicity was obvious for *P. vittata* after exposing to 2.6 mg L^{-1} CrVI for 2 w, with fronds biomass being reduced by 40% compared to the control (de Oliveira et al. 2014). Bashri et al. (2016) reported decreased growth of *Amaranthus* species after exposing to 0.52–2.6 mg L^{-1} CrVI for 7 d, attributing to high Cr accumulation in the roots at 2,620 mg kg^{-1}. Similarly, As and Cr induced stress in *P. ensiformis*. After 7 d exposure, *P. ensiformis* grew well except for plants under $AsV_{7.5}$ and/or $CrVI_{5.2}$, showing toxicity symptoms e.g., yellowing of pinna (Fig. 1a). The data suggested both 7.5 mg L^{-1} AsV and 5.2 mg L^{-1} CrVI were toxic to plants. At higher As and Cr concentrations, cell membrane and plant functionality were impacted, reducing plant biomass.

Under metal stress, accumulation of reactive oxygen species (ROS) in plants are common, leading to lipid peroxidation and membrane damage (Gill and Tuteja, 2010). Plant roots are the first organs to interact with contaminant (Huang et al. 2012), so often they show signs of toxicity first. TBARS have been used to indicate metabolic stress caused by metals including As and Cr (de Oliveira et al. 2014). In the control, TBARS in *P. ensiformis* roots were 2.1 μmol/g fw, but they increased by 35% after exposing to $AsV_{0.75}$, and 56% after exposing to $CrVI_{0.52}$ (Fig. 1b). With increasing concentrations in the media, TBARS in the roots were increased from 2.83 μmol/g fw in $As_{0.75}$ to 4.9 μmol/g fw in $As_{7.5}$ and from 3.27 μmol/g fw in $CrVI_{0.52}$ to 5.17 μmol/g fw in $CrVI_{5.2}$ (Fig. 1b), indicating additional stress to the plant.

The increased TBARS were a clear indication of oxidative stress in plants, possibly resulting from ROSs after exposure to As and Cr. The interaction of ROSs with lipids, proteins, and nucleic acids increases TBARS content and leads to ion leakage and membrane deterioration (Nahar et al. 2016). This probably occurred during reduction of AsV to AsIII and/or CrVI to CrIII (Fig. 2), a process readily occurs in plants (Kertulis-Tartar et al. 2009). The highest TBARS level (12.7 μmol/g fw) was observed in $As_{7.5}$ + $Cr_{5.2}$ treatment (Fig. 1b), consistent with the increased uptake of Evans blue dye and enhanced ion leakage from the roots (Fig. 1c, d). Increased lipid peroxidation from As or Cr mediated oxidative stress has been reported by others. For example, Das et al. (2017) observed 288% increase in TBARS level in *P. ensiformis* roots after 7 d of exposure to 3.75 mg L^{-1} As. Also, after exposing rice plants to 5.2 mg L^{-1} CrVI for 20 d in hydroponics, Cr increased both ROS and TBARS level in the plants (Zeng et al. 2011).

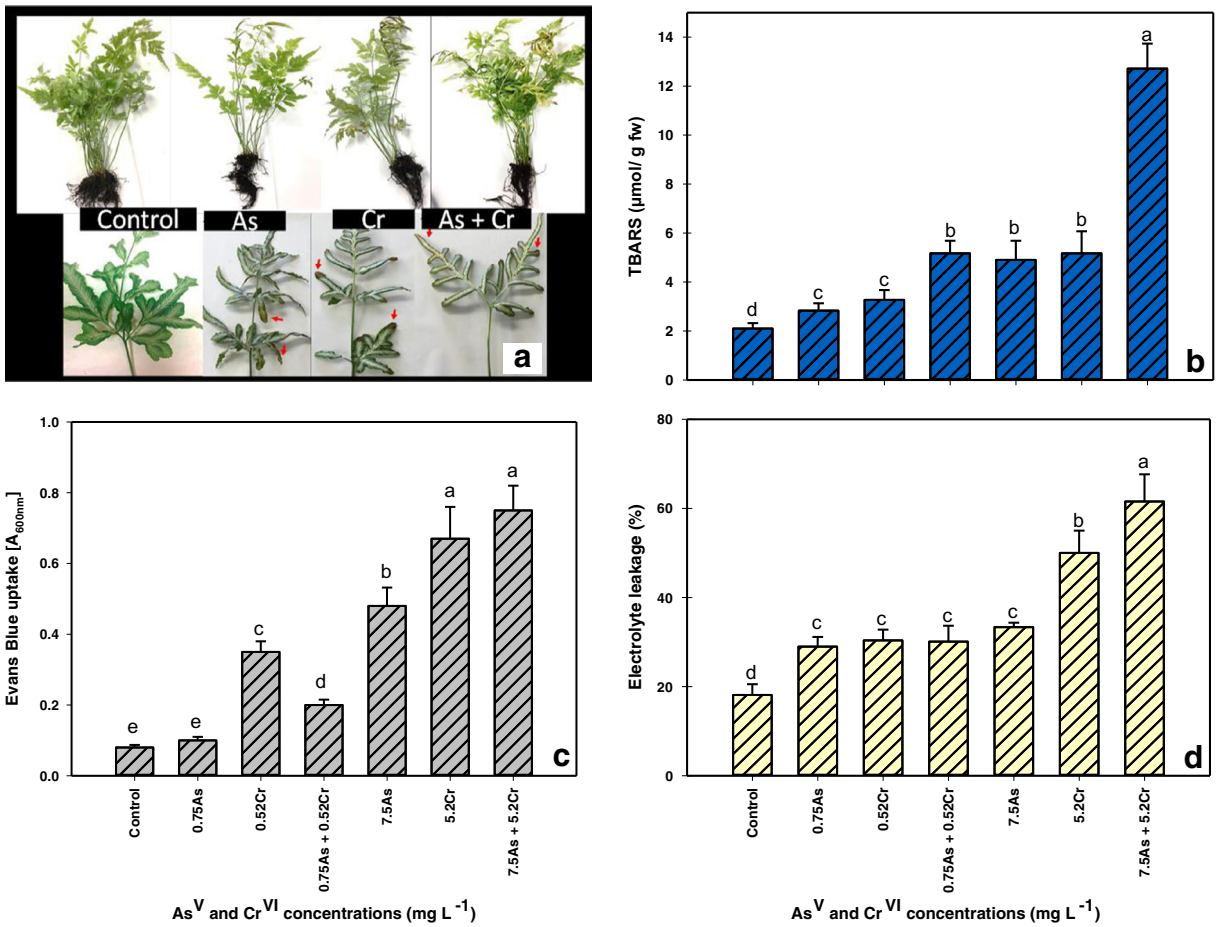

Fig. 1 Pinnae of terminal fronds of *P. ensiformis* with necrosis on the margins (**a**), and TBARS (**b**) leaf membrane injury index (**c**) and root membrane integrity (**d**) of *P. ensiformis* after growing for 7 d in 0.2 strength HS solution containing 0, 0.75, or 7.5 mg L^{-1} of AsV and 0, 0.52, or 5.2 mg L^{-1} CrVI. The bars are standard deviation of the means of three replicates. Treatments followed by the same letters are not significantly different

Membrane stability based on Evans Blue dye uptake is presented in Fig. 1c. The dye cannot penetrate through the membrane of living cells, hence a good indicator of cell death and consequently loss of root plasma membrane integrity (Das et al. 2017). The data showed that membrane injury occurred in plant roots even exposing to low levels of AsV or CrVI (Fig. 1c). The membrane injury index ($OD_{600\ nm}$) in the control was 0.08, which increased to 0.1 in $AsV_{0.75}$ and 0.35 in $Cr_{0.52}$ treatment, with Cr being 3.5 times more toxic than AsV at equal molar concentration. With increasing concentrations, the index increased to 0.48 in $As_{7.5}$ and to 0.67 in $Cr_{5.2}$ treatment (Fig. 1c), with the highest being 0.75 in the $As_{7.5}$ + $Cr_{5.2}$ treatment, consistent with its highest TBARS (Fig. 1b). Based on TBARS and membrane injury index, Cr was more toxic than As to *P. ensiformis* plants.

In addition to membrane injury based on Evans Blue dye uptake, root membrane stability based on electrolyte leakage from cells was measured. Similar to membrane injury index, the data indicated lower membrane integrity in plant roots after exposing to low levels of AsV or CrVI, accounting for 28–30% damage of roots membrane (Fig. 1d). Again, higher electrolyte leakage was observed in $CrVI_{5.2}$ than $AsV_{7.5}$ in *P. ensiformis* roots (50 vs. 33%) (Fig. 1d). The greater damage was probably due to higher Cr accumulation in *P. ensiformis* than As, i.e., 256–4161 vs. 64.7–487 mg kg^{-1} in the roots (Fig. 2). A similar increase in electrolyte leakage was observed by Pandey et al. (2009) in pea roots treated with 1.0–10 mg L^{-1} CrVI. They found that high Cr

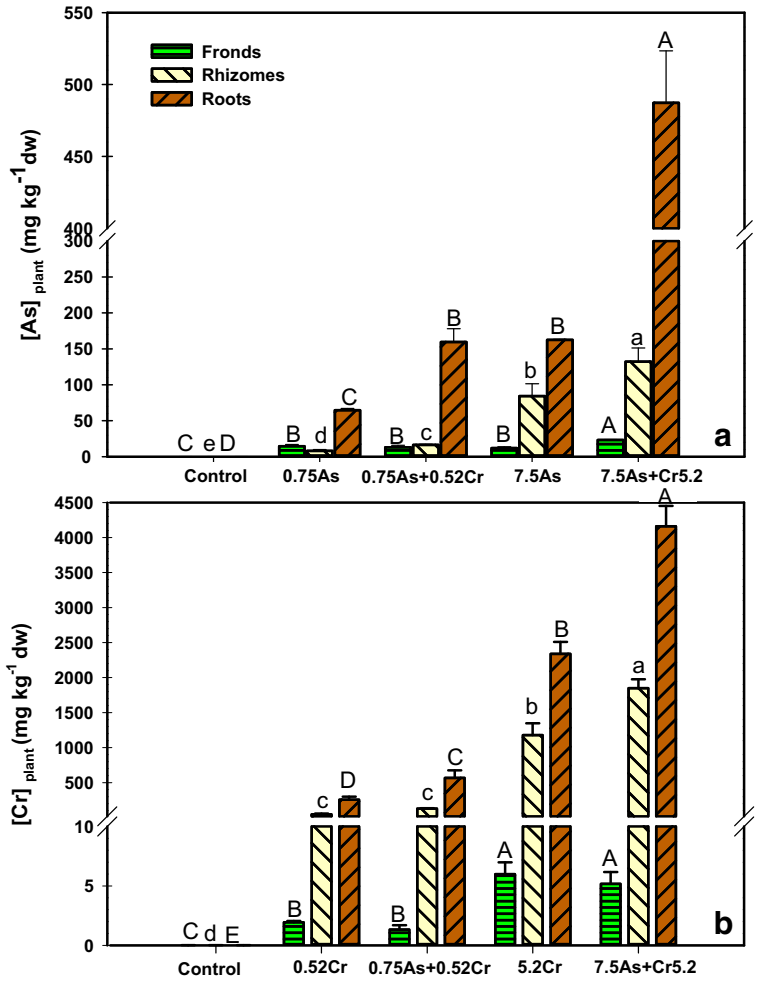

Fig. 2 Arsenic (**a**) and chromium (**b**) concentrations in *P. ensiformis* after growing for 7 d in 0.2 strength HS solution containing 0, 0.75, or 7.5 mg L^{-1} of AsV and 0, 0.52, or 5.2 mg L^{-1} CrVI. The bars are standard deviation of the means of three replicates. Treatments followed by the same letters are not significantly different

concentrations damaged plasma membrane structure in pea roots, decreasing plant growth. Our data showed that exposure of *P. ensiformis* to AsV and/or CrVI increased ROS, resulting in lipid peroxidation and membrane damage, with more damage from CrVI than AsV (Fig. 1).

Chromate induced as uptake by *P. ensiformis*, but did not change as species

To understand the impact of As on plants, it is essential to know how As is taken up and metabolized by plants. Arsenic is phytotoxic, therefore it negatively affects plant growth. Though there is no evidence it is essential for plant, growth stimulation at 20 mg kg^{-1} soil As has been reported (Kabata Pendias 2010). Tu et al. (2002) also reported moderate amounts of As enhance the growth of As hyperaccumulator *P. vittata*.

Similar to *P. vittata*, As-sensitive plant *P. ensiformis* was able to take up As and Cr from growth media. Arsenic concentrations in *P. ensiformis* increased with increasing As concentrations (Fig. 2a). For example, As concentration was 10 times higher in $AsV_{7.5}$ than $AsV_{0.75}$ in the rhizomes (8.10 vs. 84.2 mg kg^{-1}) and 2.5 times in the roots (64.7 vs. 162 mg kg^{-1}). Arsenic was mainly retained in the roots, with small amount being translocated to the fronds. Take $As_{0.75}$ for example, As concentrations in the fronds, rhizomes and roots were 14.4, 8.16 and 64.7 mg kg^{-1} (Fig. 2a), with 74% of the As being accumulated in the roots. It was probably due to complexation of AsIII by thiols, which was then sequestered in the root vacuoles. Other plants also accumulate more As in the roots. For example, 97–99% more As in the roots than shoots were observed in wheat (*Triticum aestivum*) and lettuce (Shi et al. 2017; de Oliveira et al. 2017).

The presence of CrVI promoted As uptake by *P. ensiformis* (Fig. 2a). For example, *P. ensiformis* accumulated 14.5, 8.07 and 64.7 mg kg^{-1} As in the fronds, rhizomes and roots in $As_{0.75}$ treatment, and they increased to 13.1, 16.2 and 159 mg kg^{-1} in $As_{0.75}$ + $Cr_{0.52}$ treatment. Similarly, de Oliveira et al. (2014) showed that CrVI increased As concentrations in *P. vittata* roots from 1160 to 1394 mg kg^{-1} in 3.75 AsV mg L^{-1} treatment compared to 3.75 AsV +2.6 CrVI mg L^{-1}. This is consistent with Islam et al. (2014) who observed that jute (*Corchorus olitorius*) took up 29.3–83.4 As in presence of 100 mg kg^{-1} soil As, which increased to 37.2–95.5 mg kg^{-1} in presence of 100 mg kg^{-1} As and Cr.

Since both AsV and AsIII are toxic to plants, besides total concentrations, As speciation in the growth media and plant biomass was determined (Figs. 3c and 4). Arsenic concentrations in the media decreased slightly from 7.5 to 6.0–6.7 mg L^{-1} after 7 d (Fig. 3a), with little AsV reduction to AsIII (Fig. 3c). After 7 d exposure to AsV, more AsIII than AsV was accumulated in the fronds (72–76%) whereas more AsV was accumulated in the rhizomes (56–57%) and roots (97–99%; Fig. 4a). Reduction of AsV to AsIII is a detoxification strategy for plants, probably involving the formation of phytochelatin-AsIII complex, making it inert to store in the vacuoles as a step of As detoxification in cells, which has been reported *in P. vittata* (Lei et al. 2012) and *P. cretica* (Huang et al. 2008). In *P. vittata*, AsIII reduction occurs in the rhizoid endodermis, which is common in all organisms. The presence of CrVI had little effect on As speciation in *P. ensiformis* (Fig. 4a).

Cr uptake and speciation by *P. ensiformis*

Based on increase in ROS, lipid peroxidation and membrane damage, Cr was more toxic to *P. ensiformis* than As (Fig. 1). However, compared to As, *P. ensiformis* was much more effective in taking up Cr than As (up to 4,161 mg kg^{-1}) but with <1% Cr being translocated to the fronds (Fig. 4b). This was consistent with As and Cr concentrations in the growth media, with Cr concentration decreasing by 60% and As decreasing by 19% after 7 d of growth (Fig. 3a, b). In the control, Cr contents in plants were 0.2–5 mg kg^{-1}, within the range of typical plants (Kabata Pendias 2010). In $CrVI_{0.52}$ treatment, the Cr concentrations in the fronds, rhizomes and roots were 1.94, 47.9 and 256 mg kg^{-1}, with 84% of Cr in the roots, similar to As distribution in *P. ensiformis* (Fig. 2). In $CrVI_{5.2}$ treatment, Cr concentrations in the rhizomes and roots were 1,178 and 2,339 mg kg^{-1}, which increased to 1,849 and 4,161 mg kg^{-1} in $As_{7.5}$ + $Cr_{5.2}$ (Fig. 2b).). Due to the presence of K as an ingredient of $K_2Cr_2O_7$, plants showed a positive response on Cr uptake compared to As. It seemed make sense that K was needed to alleviate Cr toxicity. Also, it was possible that cell membranes were damaged after exposing to As and Cr, thereby enhancing their permeability to transport Cr across the membrane (Tuan et al. 2008). This was supported by increased TBARS content, electrolyte leakage and membrane injury in *P. ensiformis* roots (Fig. 1). Plants may have passively taken up CrVI and AsV at phytotoxic levels through broken cell membranes. Due to its high oxidation power, CrVI causes membrane damage in plants and alters the morphological and physiological processes in plants due to ROS production (Gill et al. 2015).

As expected, *P. ensiformis* took up more Cr from growth media containing higher Cr concentrations (Fig. 2b). The Cr concentrations in the roots increased from 256 to 2,339 mg kg^{-1} from $CrVI_{0.52}$ to $CrVI_{5.2}$ treatment. The nearly 10-fold increase in Cr concentration in the roots probably indicated passive uptake of Cr by *P. ensiformis*. The Cr concentrations increased to 567 and 4,167 mg kg^{-1} in the roots in $CrVI_{0.52}$ + $AsV_{0.75}$ and $CrVI_{5.2}$ + $AsV_{7.5}$. However, As in the media did not change Cr content in the fronds (Fig. 2b). Similar to As, Cr was mostly retained in the *P. ensiformis* roots (at $CrVI_{5.2}$ + $AsV_{7.5}$ mg L^{-1}, < 1% of the Cr was transferred to the shoots). The reduced translocation could be due to different strategies to avoid toxicity in the fronds, which may include the reduction of CrVI to CrIII in the roots (Qiu et al. 2013).

Though much research has been conducted on Cr uptake by plants, it is still unclear why some plants are more efficient in Cr uptake (de Oliveira et al. 2014; Kalve et al. 2011). Rice plants accumulated large amounts of Cr in the roots (up to 2,441 mg kg^{-1}) after exposure to 7.5 mg L^{-1} CrVI for 20 d in hydroponic experiments. *P. vittata* accumulated large amounts of Cr (up to 11,370 mg kg^{-1}) in the roots after exposing to 5.2 mg L^{-1} CrVI for 14 d (de Oliveira et al. 2014). In addition, after exposing to 50 mg L^{-1} AsV and CrVI for 20 d in hydroponics, *P. vittata* accumulated much more Cr than As (11,367 vs. 585 mg kg^{-1}) (Kalve et al. 2011). Under similar condition, Indian Mustard accumulated 1,680 mg kg^{-1} Cr in the roots after exposing to 5.2 mg L^{-1} CrVI for 7 d in hydroponics, which was lower than *P. ensiformis* at

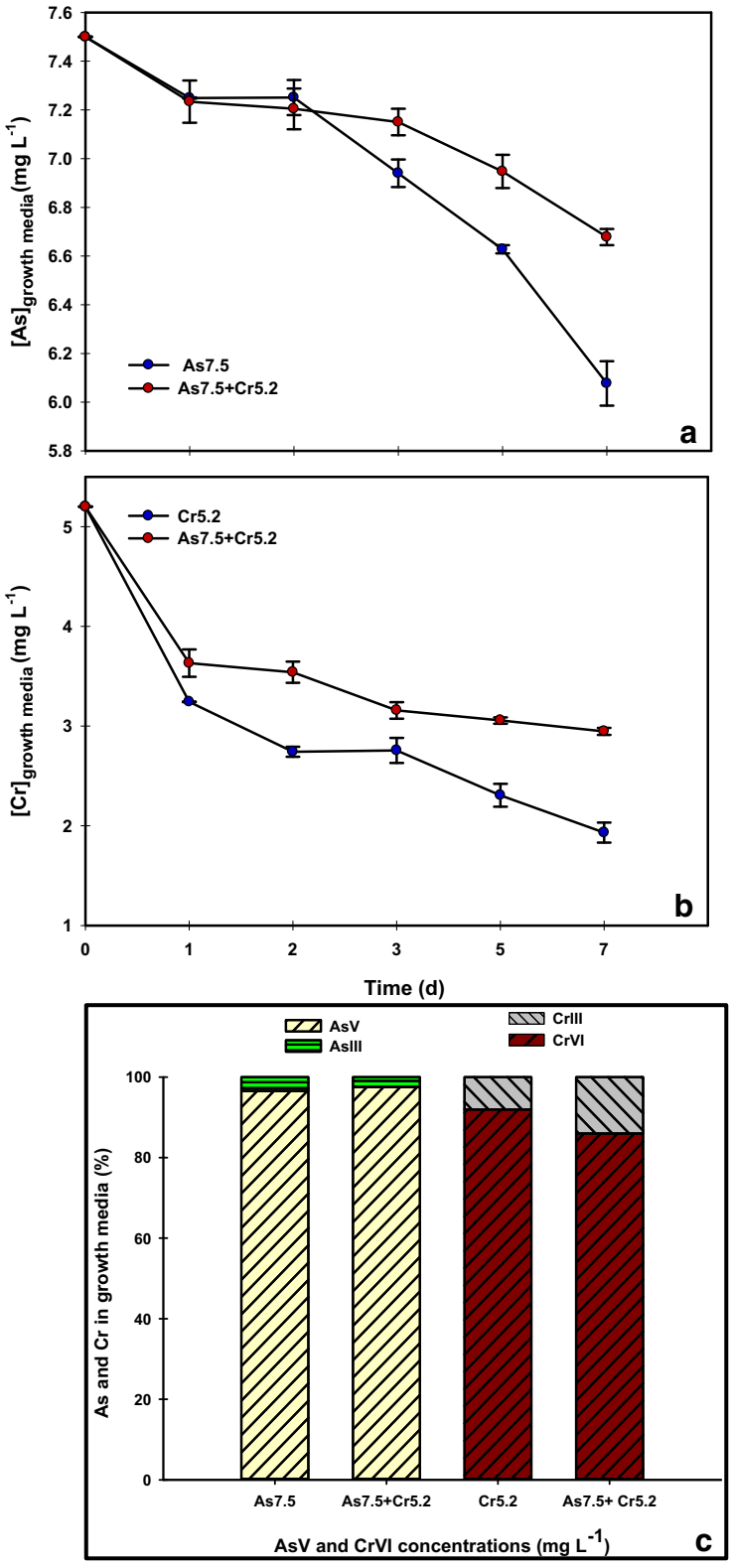

Fig. 3 Arsenic (**a**) and chromium (**b**) concentrations and speciation (**c**) in growth media of *P. ensiformis* after growing for 7 d in 0.2 strength HS solution 7.5 mg L^{-1} of AsV and/or 5.2 mg L^{-1} CrVI

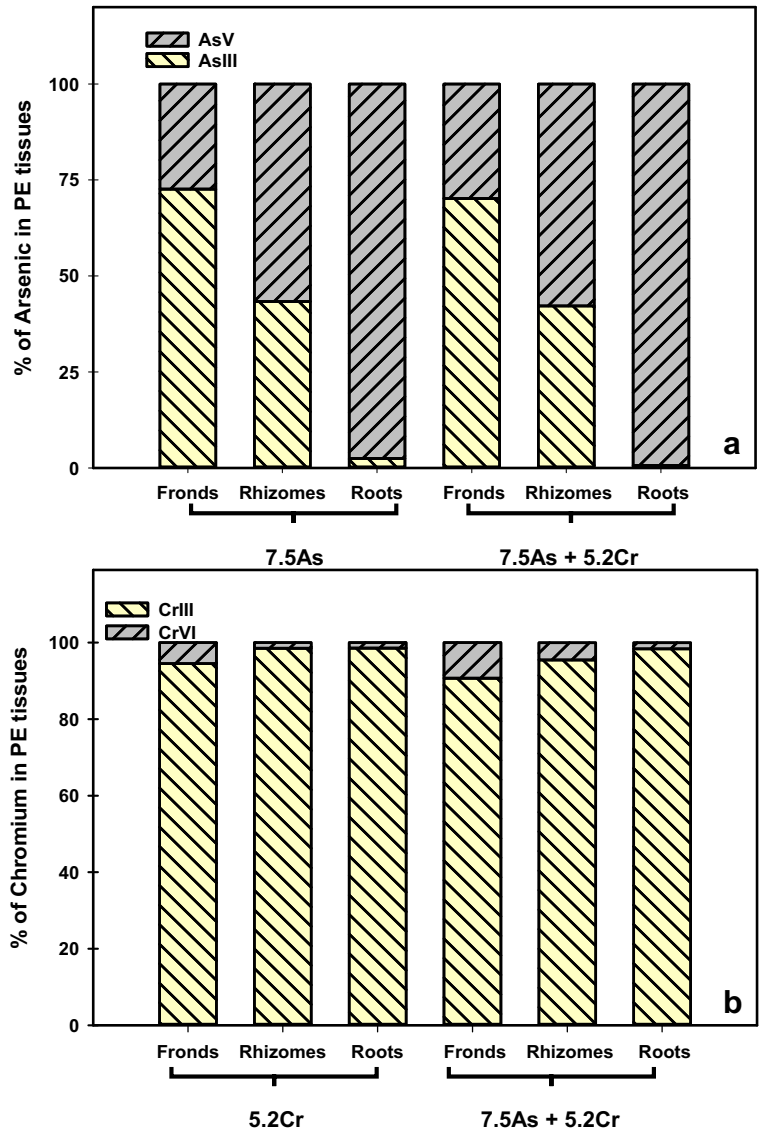

Fig. 4 Arsenic (**a**) and chromium (**b**) speciation in *P. ensiformis* after growing for 7 d in 0.2 strength HS solution containing 7.5 mg L^{-1} of AsV and/or 5.2 mg L^{-1} CrVI

4,167 mg kg^{-1}. Immobilization of Cr in the vacuoles of plant root cells is suggested as a main reason for the excessive accumulation of this metal in the roots.

To further verify that As and Cr were soluble in the solution, we calculated their solubility in 0.2 HS at fixed pH using Visual MINTEQ 3 (Gustafsson 2011). Even at the highest AsV and CrVI concentrations (7.5 and 5.2 mg L^{-1}), both elements were soluble and available for plant uptake. Predominant species in the solution included: $H_2AsO_4^-$ (98%), $HAsO_4^{-2}$ (1.1%), H_3AsO_4 (0.2%), $HCrO_4^-$ (87%), CrO_4^- (3%), and $KCrO_4^-$ (6.1%).

De Oliveira et al. (2015) did Cr speciation in the growth media after growing *P. vittata* plant for 1 d, with both Cr species remaining unchanged. In this experiment, 82–91% of the Cr in the growth media was CrVI after 7 d growth, indicating that most of the Cr taken up by *P. ensiformis* was CrVI (Fig. 3c). However, compared to As (7.5 to 6.1 mg L^{-1}), *P. ensiformis* took up more Cr, with Cr concentration in the growth media being decreased from 5.2 to 2.1 mg L^{-1} (59%) after 7 d (Fig. 3b), consistent with more uptake of Cr than As in *P. ensiformis* (306–6015 vs. 87–642 mg kg^{-1} (Fig. 2).

In addition to growth media, we also determined Cr speciation in plant biomass (Fig. 4). After uptake, Cr was transported mainly though xylem from the roots to shoots. However, when CrVI passes through the endoderm via symplast, it is reduced to CrIII, which is retained in the roots cortex cells (Hayat et al. 2012). The low amounts of CrVI in the roots suggested that *P. ensiformis* efficiently reduced CrVI to CrIII in the roots, causing more damage in the cells (Holland and Avery 2009). Though the growth media was dominated by CrVI (82–91%) (Fig. 3c), *P. ensiformis* roots were dominated by CrIII (90–98%) (Fig. 4b). After plant uptake, CrVI was immediately reduced to CrIII in carrot cells (Wu et al. 2015). Similarly, Santana et al. (2012) found only CrIII in Jagua biomass (*Genipa americana*) regardless of CrVI or CrIII was supplied in hydroponics. The CrIII in plant cells binds with phosphate of DNA, leading to cell damage and death (Choppala et al. 2012). Reduction of CrVI to CrIII occurred in the shoots of Chinese Gynura (*Gynura pseudochina*) after exposing to CrVI based on X-ray absorption spectroscopy (Cervantes et al. 2001). The CrIII was then transported via the xylem through the symplastic system, distributing in the cytoplasm of cortical cells (Mongkhonsin et al. 2011). Considering CrVI concentrations in contaminated water are within the range in the present study (0.52–5.2 mg L^{-1}), efficient CrVI uptake coupled with its low solubility in the roots suggested that *P. ensiformis* might have potential to remove Cr from contaminated water.

Conclusions

P. ensiformis plant was effective in taking AsV and CrVI, but was ineffective in translocating them to the fronds, with most of As and Cr being accumulated in the roots. Though AsV and CrVI speciation remained unchanged in the growth media, once taken up, AsV and CrVI were reduced to AsIII and CrIII in the roots, probably serving as a detoxification mechanism. While CrIII remained in the roots, more AsIII was present in the fronds and rhizomes. Accumulation of As and Cr in the roots induced oxidative stress as indicated by increased TBARS, and membrane leakage and damage. AsV and CrVI increased each other's uptake in the rhizomes and roots when co-present. Although, the mechanisms underlying the phenomenon of co-uptake of As and Cr in plants are yet to be understood.

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