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Sulfate and chromate increased each other's uptake and translocation in As-hyperaccumulator *Pteris vittata*

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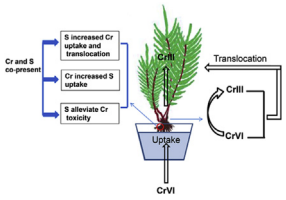
HIGHLIGHTS

- CrVI and sulfate uptake by As-hyperaccumulator *P.vittata* was investigated.
- CrVI and sulfate enhanced each other's uptake by *P. vittata*.
- *P. vittata* was efficient in CrVI accumulation in the roots with little translocation.
- Though CrVI was supplied, Cr was mainly present as CrIII in the biomass.
- CrVI was probably not taken up by *P. vittata* via sulfate transporters.

GRAPHICAL ABSTRACT



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ABSTRACT

We investigated the effects of chromate (CrVI) and sulfate on their uptake and translocation in As-hyperaccumulator *Pteris vittata*. Plants were exposed to 1) 0.1 mM CrVI and 0, 0.25, 1.25 or 2.5 mM sulfate or 2) 0.25 mM sulfate and 0, 0.5, 2.5 or 5.0 mM CrVI for 1 d in hydroponics. *P. vittata* accumulated 26 and 1261 mg kg^{-1} Cr in the fronds and roots at $CrVI_{0.1}$, and 2197 and 1589 mg kg^{-1} S in the fronds and roots at $S_{0.25}$. Increasing sulfate concentrations increased Cr root concentrations by 16–66% and helped CrVI reduction to CrIII whereas increasing CrVI concentrations increased frond sulfate concentrations by 3–27%. Increasing sulfate concentrations enhanced TBARS concentrations in the biomass, indicating oxidative stress caused lipid peroxidation in plant cell membranes. However, addition of 0.25–2.5 mM sulfate alleviated CrVI's toxic effects and decreased TBARS from 23.5 to 9.46–12.3 μmol g^{-1} FW. Though CrVI was supplied, 78–96% of CrIII was in the biomass, indicating efficient CrVI reduction to CrIII by *P. vittata*. The data indicated the amazing ability of *P. vittata* in Cr uptake at 289 mg kg^{-1} h^{-1} with little translocation to the fronds. These results indicated that *P. vittata* had potential in Cr phytoremediation in contaminated sites but further studies are needed to evaluate this potential. The facts that CrVI and sulfate helped each other in uptake by *P. vittata* suggest that CrVI was not competing with sulfate uptake in *P. vittata*. However, the mechanisms of how sulfate and CrVI enhance each other's accumulation in *P. vittata* need further investigation.

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1. Introduction

Chromium (Cr) has gained environmental importance due to its toxic effects on living organisms. Though it occurs naturally in soils, anthropogenic activities are major sources of Cr contamination, including pigment-electroplating industries, mining processes and fertilizer applications (Kristine et al., 2013). The health effects of human exposure to Cr range from dermatitis and dermatosis to various cancers. Cr exposure is also associated with decreases in plant growth and changes in plant morphology (López-Bucio et al., 2014).

Both hexavalent (CrVI) and trivalent Cr (CrIII) can be found in soils, however, they differ significantly in toxicity. While CrVI is mobile and usually present as chromate (CrO_4^{-2}) or dichromate ($Cr_2O_7^{-2}$) in soils, CrIII is often present as oxides and is less mobile. In humans, CrVI is classified as a carcinogen of Group A (ATSDR, 2012) while CrIII is an essential nutrient (Dimitroula et al., 2015). The USEPA limit for total Cr in potable water and discharge to surface water is 0.05 and 0.1 mg L^{-1}, respectively (EPA, 1990). The differences in Cr's behaviors make it important to understand Cr speciation in the environment. Unlike Cr, sulfur is an essential macronutrient for plant growth, often making up 0.5–1.0 g kg^{-1} of plant's biomass (Marschner, 2012). Its main form for plant uptake is sulfate, which is taken up by the roots through sulfate transporters (Marschner, 2012).

Due to CrVI's toxicity, the impact of Cr on plants has been a subject of extensive study. Unlike sulfur, Cr is not an essential element, so plants would not have likely developed a specific system for its uptake (Shanker et al., 2005; Oliveira et al., 2012). Plant uptake of CrIII is a passive process with no energy being required (Shanker et al., 2005). However, CrVI is likely taken up by plants through an active mechanism via sulfate or phosphate transporter because CrVI structurally resembles sulfate and phosphate (Kim et al., 2006). Competitive uptake between sulfate and CrVI has been shown in plants possibly because CrVI uses sulfate transporter due to their structural similarity (Ramírez-Díaz et al., 2008). It has been shown that sulfate inhibits CrVI uptake in wheat and barley (Kleinman and Cogliatti, 1997). However, other researchers reported that sulfate has no effect on plant CrVI uptake (Zakaria et al., 2007). In addition, CrVI also inhibits sulfate uptake although a general effect of CrVI on active membrane transport has been excluded (Schiavon et al., 2007). However, CrVI uptake was stimulated after pre-cultivation of *Zea mays* plants in sulfate-limited nutrient media, suggesting that other transporters could be involved in CrVI uptake in plants (Schiavon et al., 2007). In *Pteris vittata* plants, CrVI inhibited phosphate uptake when CrVI was supplied after 1-d exposure (de Oliveira et al., 2015). Similarly, Qian et al. (2013) found that increasing P concentration in the medium alleviated Cr toxicity in alga *Chlorella vulgaris* by decreasing its Cr absorption. The authors attributed the reduced metal toxicity to decreased Cr absorption under high-P conditions.

P. vittata is efficient in arsenic (As) accumulation (Ma et al., 2001) and can also accumulate large amounts of Cr in the roots (de Oliveira et al., 2014; de Oliveira et al., 2015; Sridhar et al., 2011; Kalve et al., 2011). Some researchers speculate that CrVI is probably taken up by *P. vittata* via sulfate transporters (Shiavon et al., 2007, 2012). However, little is known about the impact of sulfate on CrVI uptake and translocation in *P. vittata*. The overall objective of this study was to evaluate the impact of sulfate (essential nutrient) and CrVI (toxic element) on each other's uptake and translocation in *P. vittata*, following exposure to CrVI and sulfate at different concentrations for 1 d in aerated hydroponic solutions. Our objectives were to 1) investigate the effects of sulfate and CrVI on each other's uptake and translocation in *P. vittata*, and 2) determine CrVI speciation in PV fronds and roots. Knowledge of how *P. vittata* takes up and transports Cr provides insight into Cr uptake in other plants.

2. Material and methods

2.1. Plant material and growth conditions

Six-month old *P. vittata* plants of uniform size with 4–5 fronds were cultivated in our laboratory. The plants were acclimatized in 0.2-strength Hoagland solution (HS) at pH 5.7 with 1 mM KOH–MES buffer for 4 weeks under continuous aeration. The acclimated plants were transferred to 1 L opaque containers containing S-free 0.2X HS for 1 week. The water loss via transpiration was replenished by frequent additions of deionized water. Plants were then transferred to 0.2X HS, which were spiked with different concentrations of CrVI as $K_2Cr_2O_7$ and sulfate as Na_2SO_4, specifically 1) 0.25 mM sulfate and 0, 0.5, 2.5 or 5.0 mM CrVI or 2) 0.1 mM CrVI and 0, 0.25, 1.25 or 2.5 mM sulfate. They are referred to as $CrVI_{0.5}$, $CrVI_{2.5}$, or $CrVI_{5.0}$, and $S_{0.25}$, $S_{1.25}$, or $S_{2.5}$. CrIII as $Cr(NO_3)_3$ $9H_2O$ was tested with 0.1 mM CrIII and 0 or 1.25 mM sulfate. In addition, we also tested the Cr uptake by another fern *Adiantum capillus,* which was grown for 1 d in 0.2-strength HS containing 0.25 mM sulfate and 0, 0.5, 2.5 or 5.0 mM CrVI.

The plants were kept in a controlled environment with 8 h photoperiods at light intensity of 350 μmol m^{-2} s^{-1}, 28/23 °C day/night temperature, and 60–70% relative humidity. Plants were harvested 1 d after the treatments.

2.2. Plant tissues analysis

Following 1 day exposure to CrVI and sulfate, the *P. vittata* plants were separated into aboveground (fronds) and belowground (roots and rhizomes) biomass. To remove sorbed Cr from the roots, *P. vittata* roots were washed with distilled water, ice-cold phosphate buffer (1 mM Na_2HPO_4, 10 mM MES and 0.5 mM $Ca(NO_3)_2$, pH 5.7), and again with distilled water.

Oven-dried (65 °C for 2 days) samples were digested with HNO_3 and H_2O_2 for Cr, sulfur (S) and potassium (K) analysis on a hot block digester (Environmental Express, Mt. Pleasant, SC) using USEPA Method 3050B (de Oliveira et al., 2015). Total Cr, S and K concentrations in digested solution were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS Perkin–Elmer Corp., Norwalk, CT). Internal standard has been used and standard solution at 20 μg L^{-1} Cr was measured every 20 samples to monitor the stability of ICP-MS. To determine the amount of Cr precipitated on *P. vittata* root surfaces, plant roots (~0.2 g) were washed in 50 ml 1:1 v/v HNO_3 and water for 20 min and the Cr concentrations in the supernatant was determined using ICP-MS.

2.3. Cr speciation in plant and Hoagland solution

In this study, a modified method of alkaline digestion based on USEPA Method 3060A (US Environmental Protection Agency, 1995) was employed to extract CrVI in plants. 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. The solution was used to determine CrVI by ICP-MS. During the extraction, CrVI was solubilized by Na_2CO_3 solution to form Na_2CrO_4 while CrIII species formed insoluble hydroxides or carbonates.

The accuracy of Cr speciation using the procedure was determined by spiking 10 mg L^{-1} CrIII or CrVI to Cr-free plant extract. There was no change in redox status since the recovery of the

spiked Cr was 100 ± 5% (data not shown). 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.

In addition to Cr speciation in plant biomass, CrVI concentrations in 0.2X HS were measured using a colorimetric reagent specific for CrVI, 1,5-diphenylcarbazide, which was dissolved in 0.05% acetone (Eaton et al., 1995). Absorbance was measured with spectrophotometer (UVI1800U, Shimadzu Corp., Columbia, MD) at 540 nm, and CrVI concentration was calculated with a standard curve prepared using a series of CrVI dilutions (1–25 mg L^{-1}). Furthermore, metal speciation in solution was performed using Visual MINTEQ3 (Gustafsson, 2011).

2.4. Lipid peroxidation in plant biomass

The amount of thiobarbituric acid reactive substances (TBARS) was determined following Groppa et al. (2001) to determine the impact of Cr on lipid peroxidation in *P. vittata* fronds. About 0.3 g of frozen-tissues was cut into small pieces and homogenized, using a cold mortar and pestle in ice bath with 1.5 ml of 5% (wt/v) trichloroacetic acid (TCA) solution. The homogenate was transferred into fresh tubes and centrifuged at 10,000 × g for 10 min at room temperature. To 1 ml of the aliquot of the supernatant, 1 ml of 20% (w/v) TCA containing 0.5% (w/v) TBA were added. The mixture was heated at 95 °C for 30 min and then quickly cooled on ice. The absorbance of the supernatant was measured at 532 nm and 600 nm. The TBARS content was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} based on fresh biomass.

2.5. Statistical treatments

All treatments were replicated three times. All concentrations were expressed on a dry weight basis except TBARS. Significant differences were determined by using one–way analysis of variance (ANOVA) and treatment means were compared by Tukey's multiple range tests at $p < 0.05$ was performed using JMP 10 PRO (SAS Institute Inc., Cary, NC, 1989–2010). Associations between variables were also assessed using Pearson's correlation coefficient.

3. Results and discussion

In our experiment, the effects of CrVI and sulfate on each other's uptake and translocation in *P. vittata* were tested by exposing *P. vittata* for 1 d in 0.2X Hoagland solutions (HS) containing 0.25 mM sulfate and 0, 0.5, 2.5 or 5.0 mM CrVI or 0.1 mM CrVI and 0, 0.25, 1.25 or 2.5 mM sulfate. In addition, to evaluate CrIII uptake by PV, 1 d experiment with 0.1 mM CrIII and 0 or 1.25 mM sulfate was also carried out.

Exposing plants to high metal concentrations in hydroponics can be misleading if metals precipitate in solution and become unavailable to plants. Therefore, we calculated the solubility of Cr and sulfate in 0.2X HS at fixed pH using Visual MINTEQ 3 (Gustafsson, 2011). Even at the highest CrVI and sulfate concentrations, both elements were soluble for plant uptake. Predominant species in the solution included: $HCrO_4^-$ (74%), and $Cr_2O_7^{-2}$ (24%) for CrVI, and SO_4^{2-} (81%), $CaSO_4^+$ (11%), KSO_4^+ (2.6%) and $MgSO_4^+$ (4.4%) for sulfate.

3.1. Sulfate enhanced Cr uptake and translocation by P. vittata

CrVI was readily taken up by *P. vittata* roots, which was mainly retained in the roots with little translocation to the fronds. For example, at $CrVI_{0.5}$, Cr concentrations were 52.2 and 6940 mg kg^{-1} Cr in *P. vittata* fronds and roots (Fig. 1A), with 99% of the Cr being accumulated in the roots. At $CrVI_{2.5}$ and $CrVI_{5.0}$, the fronds and roots Cr increased to 296–367 and 11,913–16,014 mg kg^{-1}, respectively (Fig. 1A), again with Cr mainly being in the roots, similar to other plants. Shanker et al. (2005) speculated that Cr accumulation in the roots may be due to 1) Cr precipitation as insoluble salts, 2) immobilization with molecules such as sugar, pectins, celluloses, and hemicelluloses or 3) compartmentalization in the vacuoles of root cells. With increasing external Cr concentrations, Cr accumulation in the fronds increased 5.7–7 fold, which was more than the 1.7–2.3 fold for the roots, indicating limited Cr translocation from the roots to the fronds (Fig. 1AB). Compared to solution Cr concentration at 0.5 mM (26 mg L^{-1}), the root Cr concentration was 267-fold greater after 1 day uptake. The data indicated the amazing ability of *P. vittata* in Cr uptake at 289 mg kg^{-1} h^{-1}.

To test if efficient Cr uptake is unique in *P. vittata*, we tested Cr uptake by fern plant *A. capillus.* Similar to *P. vittata*, *A. capillus* was efficient in taking up CrVI, with corresponding Cr concentrations being 6,080, 9050 and 13,390 mg kg^{-1} in the roots at $CrVI_{0.5}$, at $CrVI_{2.5}$ and $CrVI_{5.0}$, which were 14–32% less than those in *P. vittata* roots (Fig. 2A). Compared to the growth media, the amounts of Cr in plants roots were 51–267 fold greater than those in the media for both fern plants. It seemed that efficient Cr uptake also occurred in other fern plants. However, further study is needed to confirm this observation.

It is known that Cr negatively affects plant growth (Kabata–Pendias, 2011). There is no evidence that CrVI is essential for plant growth, although growth stimulation at low CrIII concentrations at 0.05–1 mg L^{-1} has been reported for some plants (Peralta-Videa et al., 2009). After 1 d exposure, *P. vittata* did not show sign of Cr toxicity in this study (data not shown). However, Cr toxicity was obvious under longer exposure of 2 weeks to 0.1 mM CrVI, with *P. vittata* biomass being reduced from 3.37 to 1.34 g, a 60% reduction (de Oliveira et al., 2014). The data suggested that CrVI is toxic to *P. vittata* at > 0.1 mM (5.2 mg L^{-1}) under long term exposure. Compared to other plants, *P. vittata* plants were much more efficient in accumulating Cr in the roots (up to 16,014 mg kg^{-1}, Fig. 1A). The reason for its high accumulation in the roots could be because CrVI is immobilized there, rendering it non-toxic to the plant. Since CrVI must cross the endodermis via symplast, CrVI was probably reduced to CrIII in *P. vittata* roots, which reduced its mobility from the roots to fronds and corresponded to its low translocation. It has been reported that CrIII bound with oxalate, citrate malate and acetate ligants in plants, inhibiting its translocation (Aldrich et al., 2003). Compared to other plants, the ability of *P. vittata* to concentrate Cr in the roots was extraordinary since Cr concentration in its roots was much higher than that reported for the most efficient Cr-accumulator plants (de la Rosa et al., 2014; Schiavon et al., 2007). To determine if the Cr was accumulated on the roots surface, the roots were washed with HNO_3 (1:1 v/v) for 20 min. Most of the Cr was inside the roots (89–98%) (data not shown).

It is possible that plants passively take up large amounts of CrVI at phytotoxic levels through broken cell membranes. For example, CrVI causes plasmolysis in peripheral cells on root surfaces of bush beans (Aldrich et al., 2003). Thiobarbituric acid reacting substances (TBARS) have been used to indicate metabolic stress caused by metals including Cr (Ercal et al., 2001). It is an indicator of free radical formation in the tissues and is used as an index of lipid peroxidation in plants (Hartley-Whitaker et al., 2001). In the absence of Cr, the TBARS in *P. vittata* fronds were 9.44 μmol g^{-1} fw (Fig. 1C). With increasing external CrVI concentrations, TBARS increased to 23.7–58.8 μmol g^{-1} fw. The increase in TBARS was 150–522% in the fronds, a clear indication of oxidative stress in *P. vittata* plants. The increased concentrations of TBARS in the

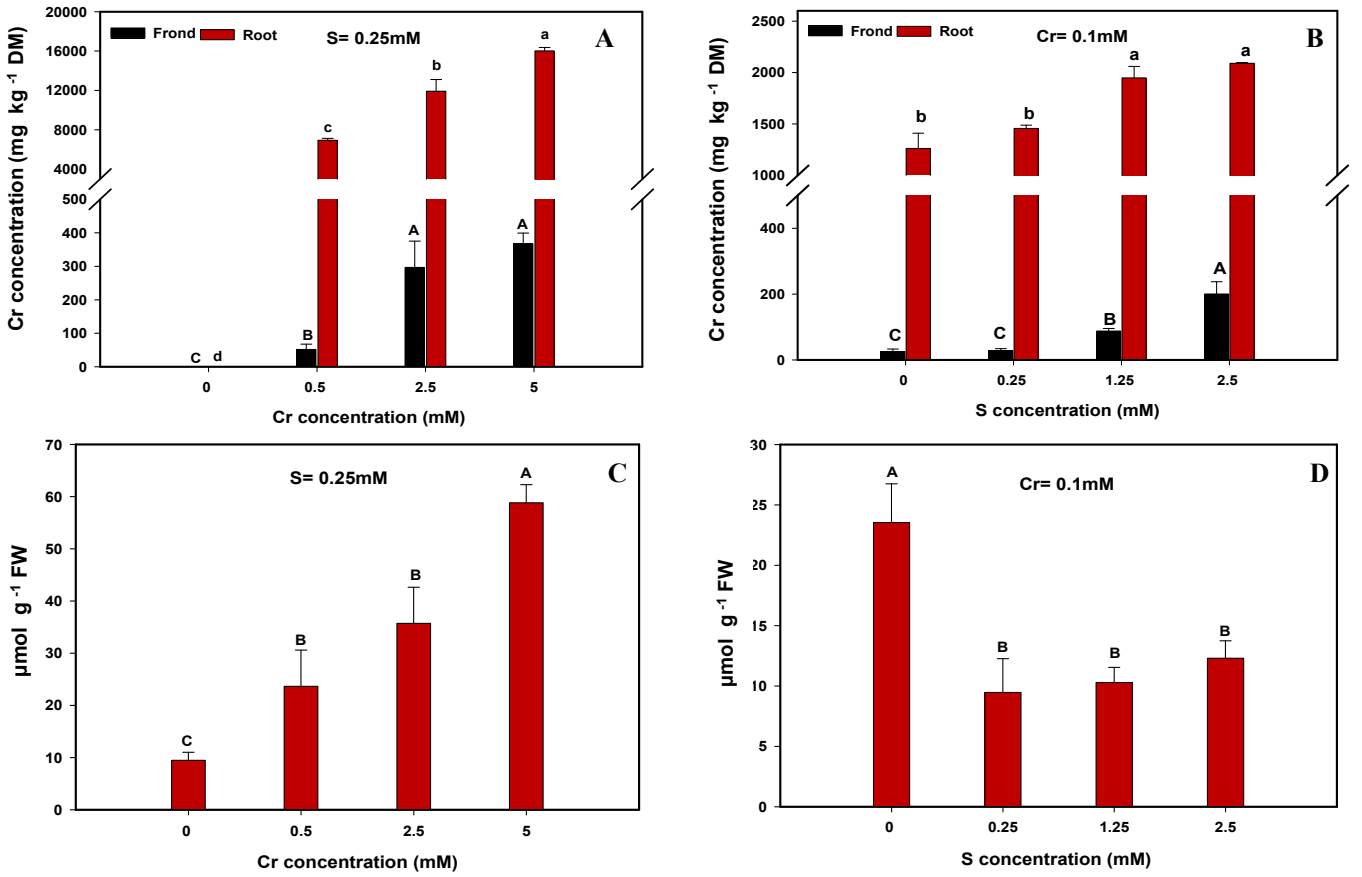

Fig. 1. Effects of CrVI and sulfate on Cr concentrations (AB) and lipid peroxidation (CD; µmol malondialdehydeg^{-1} fw) in the fronds and roots of *P. vittata* after growing for 1 d in 0.2-strength Hoagland solution containing 0.25 mM sulfate and 0, 0.5, 2.5 or 5.0 mM CrVI, and 0.1 mM CrVI and 0, 0.25, 1.25 or 2.50 mM sulfate. The bars are standard error of the means of three replicates. Treatments followed by the same letters are not significantly different at $\alpha = 0.05$.

biomass indicated lipid peroxidation of cell membranes in plants caused by oxidative stress (Flora, 2011). This is because production of reactive oxygen species results in the blockage or inactivation of various enzymes and functional groups that regulate the normal functioning of plants (La Rocca et al., 2009). However, addition of 0.25–2.5 mM sulfate decreased TBARS concentration from 23.5 to 9.46–12.3 µmol g^{-1} fw, indicating that sulfate alleviated Cr-induced oxidation stress in *P. vittata* fronds (Fig. 1D).

It was unclear why addition of sulfate increased Cr uptake by *P. vittata*. At 0.1 mM CrVI, *P. vittata* averaged 25.7 and 1261 mg kg^{-1} Cr in the fronds and roots, with 98% of the Cr being in the roots (Fig. 1B). At 0.1 mM CrVI and 0.25 mM S, *P. vittata* accumulated 28.8 and 1457 mg kg^{-1} Cr in the fronds and roots, respectively (Fig. 1B). As external sulfate concentration increased from 2.5 to 5.0 mM, Cr in *P. vittata* roots increased from 1947 to 2090 mg kg^{-1} (Fig. 1B). However, Cr concentrations in the fronds increased only slightly from 87.7 to 200 mg kg^{-1} Cr. We demonstrated that the addition of sulfate to the growth media significantly increased Cr uptake by *P. vittata*, with the increase occurring mostly in the roots.

CrVI is taken up by some plants via sulfate or phosphate transporters (Shiavon et al., 2012; de Oliveira et al., 2015; Shardendu, 2013; Qian et al., 2013). Schiavon et al. (2007) tested the impact of sulfate on CrVI or phosphate uptake in maize plants. After 2 day exposure, they found that CrVI inhibits sulfate uptake but not phosphate uptake. The fact that sulfate at 1.25–2.5 mM, which was 12.5–25 times greater than CrVI at 0.1 mM, enhanced Cr uptake in *P. vittata* by 1.3–7.8 fold (Fig. 1B) may suggest that Cr didn't not share the same transporters with sulfate in *P. vittata*, which is different from other plants (Schiavon et al., 2012). Kim et al. (2006) reported that the overexpression of putative yeast transcriptional activators MSN1 and NtST1 (*Nicotiana tabacum* sulfate transporter 1) enhanced Cr and sulfate accumulation in transgenic tobacco. These researchers suggested that both sulfate and CrVI are taken up via sulfate transporters in plants. However, de la Rosa et al. (2014), working with *Helianthus annuus* seedlings in hydroponics solution containing 0.19 mM CrVI and 1 mM sulfate, found that after 15 days of exposure, sulfate increased Cr accumulation in the roots by 29% and in the shoots by 66% compared to control. The authors attributed that it may be possible that plant age plays a role in the amount of expressed sulfate transporters. de Oliveira et al. (2014) found that, after 1 week exposure to 0.05 mM CrVI and 1.25 mM sulfate, sulfate increased Cr concentration by 4-fold to 841 mg kg^{-1} in the fronds and 3-fold to 14,473 mg kg^{-1} in the roots compared to the control. In the present study, addition of sulfate to *P. vittata* plants increased Cr uptake in the roots and fronds besides alleviation of Cr-induced oxidative stress (Fig. 1AB).

3.2. CrVI was reduced to CrIII in the roots of P. vittata

To better understand the impact of Cr on plant uptake, it is important to know their speciation. After 1 day exposure, Cr speciation in *P. vittata* growth media containing 0.25 mM sulfate and 0.1, 2.5 or 5.0 mM CrVI revealed limited CrVI reduction (7–11%) (Fig. 3A), indicating that most of the Cr taken up by *P. vittata* was CrVI (93–89%). These results are in agreement with McGrath (1982) who showed that, after culturing oat plants for 4 weeks,

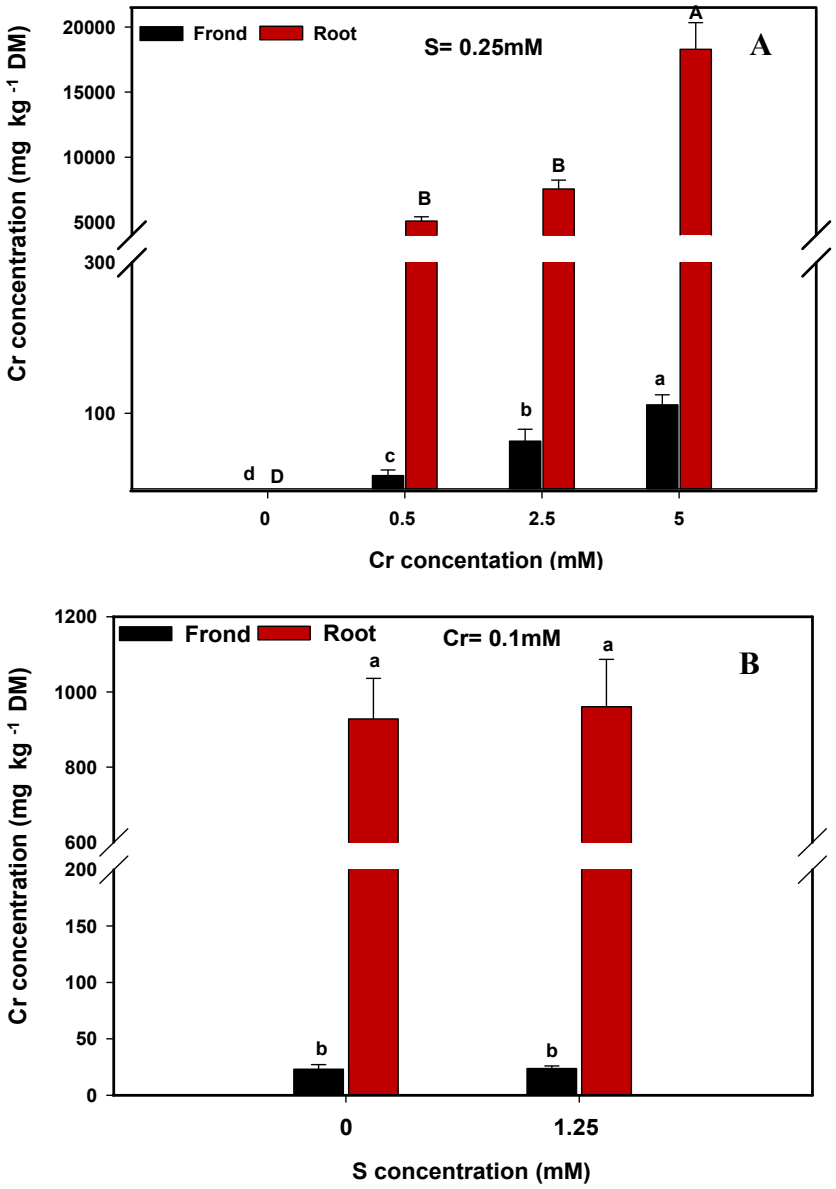

Fig. 2. Effects of CrVI and sulfate on Cr concentrations in the fronds and roots of *Adiantum capillus* after growing for 1 d in 0.2-strength Hoagland solution containing 0.25 mM sulfate and 0, 0.5, 2.5 or 5.0 mM CrVI (A) and effects of CrIII and sulfate on Cr concentrations in the fronds and roots of *P. vittata* after growing for 1 d in 0.2-strength Hoagland solution containing 0.1 mM CrIII and 0 or 1.25 mM sulfate (B). The bars are standard error of the means of three replicates. Treatments followed by the same letters are not significantly different at $\alpha = 0.05$.

Cr species remain unchanged in the nutrient solution containing CrIII or CrVI.

In addition to Cr speciation in growth media, we also determined Cr speciation in the biomass. Though the plants were primarily exposed to CrVI, CrIII was the main form in *P. vittata* roots, i.e., the solution was dominated by CrVI (89–93%; Fig. 3A), but *P. vittata* roots were dominated by CrIII (89–96%; Fig. 3B). At 0.5–5.0 mM Cr, *P. vittata* accumulated 52–367 and 6940–16,015 mg kg^{-1} of Cr in the fronds and roots, with 64–78% and 89–96% of the Cr as CrIII in the fronds and roots, respectively (Fig. 3B). The low amounts of CrVI in the roots suggested that *P. vittata* probably reduced toxic CrVI to relatively nontoxic CrIII. It was possible that CrVI was reduced to CrIII on the root surface by *P. vittata*, which is rich in microorganism (Cheung and Gu, 2007). It was also possible that CrVI was reduced to CrIII in the rhizomes similar to As (Mathews et al., 2010). Since the rhizomes were not separated from the roots in this study, further research is needed to identify the tissue location and nature of CrVI reduction.

Reduction of CrVI to the less toxic and less mobile CrIII is probably an important step in Cr detoxification in plants including *P. vittata*. Some plants have the ability to reduce CrVI to CrIII in the roots (Santana et al., 2012). In *P. vittata* roots, 89–93% of the Cr was present as CrIII compared to 64–78% in the fronds (Fig. 3B). In other words, much higher amount of CrVI was translocated to the fronds compared to CrIII. This was probably because CrVI was more soluble than CrIII. This hypothesis was tested by shaking 0.5 g of *P. vittata* roots with 10 mL DI water for 2 h, though the roots were dominated by CrIII, 93–96% of the Cr in the solution was CrVI (Fig. 3C). As little CrIII was detected in the solution, the data suggested that CrIII in the roots was insoluble.

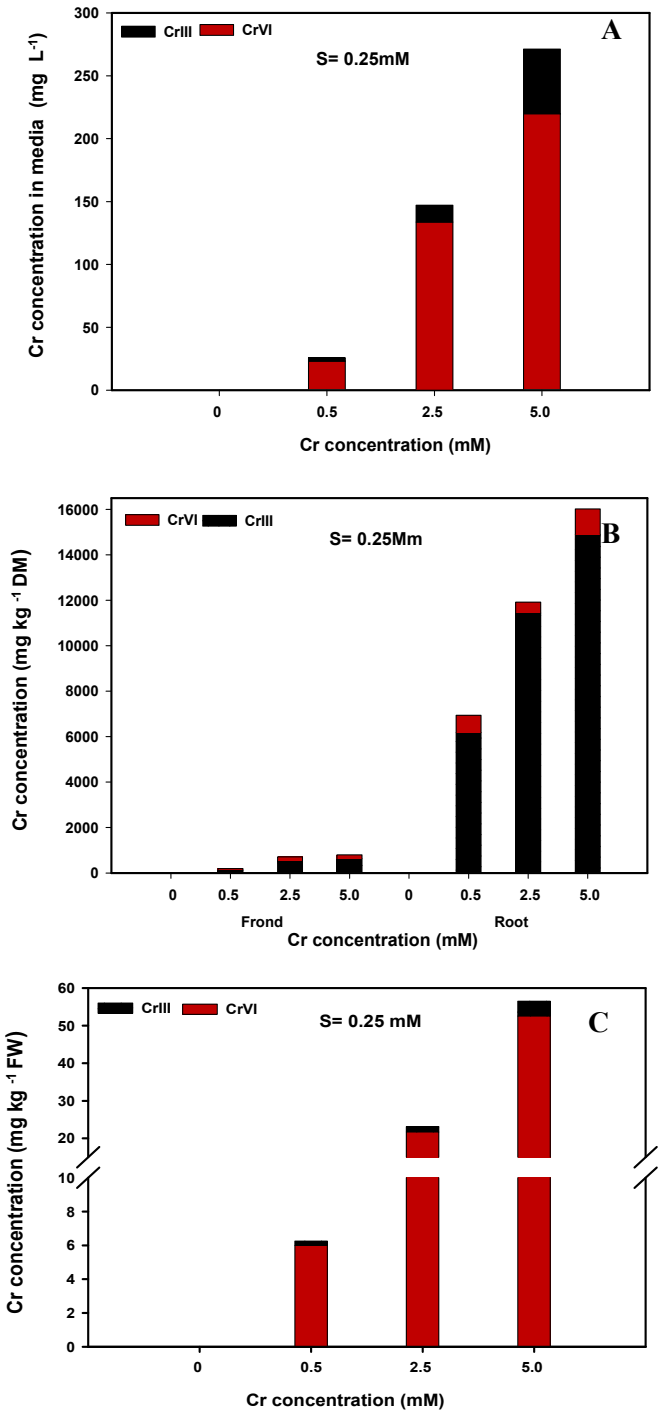

Fig. 3. Cr speciation in 0.2-strength Hoagland solution (HS) (A), in the fronds and roots of *P. vittata* (B), and in solution after shaking 0.5 g of *P. vittata* roots with 10 mL DI water for 2 h (C). *P. vittata* was grown for 1 d in 0.2-strength HS containing 0.25 mM S and 0, 0.5, 2.5 or 5.0 mM CrVI. The bars are standard error of the means of three replicates. Treatments followed by the same letters are not significantly different at $\alpha = 0.05$.

To compare *P. vittata* uptake of CrVI with CrIII, we evaluated CrIII uptake by *P. vittata* after 1 day growth in 0.2 X HS. At $CrIII_{0.1}$, *P. vittata* accumulated 928 mg kg^{-1} Cr in the roots and in the presence of 1.25 mM sulfate, Cr concentration was 960 mg kg^{-1} in the roots (Fig. 2B). In comparison, at $CrVI_{0.1}$, *P. vittata* accumulated 1261 mg kg^{-1} in the roots (Fig. 1B). The data indicated that *P. vittata* was much more efficient in taking up CrVI than CrIII, resulting in 1.4-fold higher Cr uptake with CrVI than CrIII (Figs. 1B and 2B). Sulfate did not affect CrIII uptake, indicating that CrIII did not depend on metabolic energy to be taken up by *P. vittata*, which is in agreement with previous experiments (Shanker et al., 2005). The fact that the growth solution was dominated by CrVI (89–93%) and *P. vittata* was more efficient in taking up CrVI suggested that CrVI was taken up by *P. vittata* and was probably reduced in the roots. At $CrVI_{2.5}$, *P. vittata* accumulated 74% and 96% of CrIII in the fronds and roots respectively (Fig. 3B), which was consistent with Cr_2O_3 (chromium oxide) being detected in the roots using X-ray diffraction (data not shown). Similar results were reported in sunflower (*H. annuus*) (de la Rosa et al., 2014) who determined Cr speciation in the roots using micro XRF after exposing the plant to CrVI and sulfate in hydroponics (0.19 mM CrVI and 0, 0.1, 1 or 10 mM sulfate). They revealed that CrVI is rapidly reduced to CrIII in the roots and 80% is precipitated as CrIII phosphate. Considering CrVI concentrations in contaminated water are within the range in the present study (0.1–5.0 mM) (EPA, 1990), *P. vittata* not only removed CrVI from solution (289 mg kg^{-1} h^{-1}), but also decreased Cr toxicity by reducing CrVI to CrIII, suggesting that *P. vittata* may have potential to remove Cr from contaminated water.

3.3. Addition of CrVI increased S concentrations in *P. vittata*

Sulfur requirement for optimal plant growth varies from 1000 to 5000 mg kg^{-1} in plants (Marschner, 2012). The sulfate concentrations in *P. vittata* fronds and roots were within the range reported for *P. vittata* plants (Wei et al., 2010). In the presence of 0.25 mM sulfate, sulfur concentration in *P. vittata* fronds and roots were 2176 and 1166 mg kg^{-1}, with 1.9 times more sulfur being in the fronds than in the roots (Fig. 4A). Sulfur translocation to the fronds is typical for macronutrients in plants, with more being in aerial parts. However, it was unclear why increasing CrVI concentration in the solution increased fronds and roots S concentration in *P. vittata* ($p < 0.05$). For example, at 2.5–5.0 mM CrVI, S concentrations were increased by 29–10% from 2176 to 2399–2798 mg kg^{-1} in the fronds and by 36–57% from 1165 to 1836–1585 mg kg^{-1} in the roots (Fig. 4B). *P. vittata* was effective in translocating sulfur in the presence of Cr, with most of the sulfur in the fronds (Fig. 4B), suggesting that *P. vittata* probably took up more sulfur in respond to Cr accumulation. In other words, sulfur uptake in *P. vittata* corresponded to Cr uptake rather than on the sulfate level in the medium (Fig. 4AB). The fact that CrVI did not decrease sulfur concentrations in *P. vittata* (Fig. 1AB) may be because sulfur was taken up by *P. vittata* more rapidly than CrVI by the roots. The fact that CrVI did not affect sulfur concentrations suggested that sulfate probably did not compete with CrVI uptake and translocation or *P. vittata* possessed unique sulfate transporters that did not compete with CrVI uptake. Different sulfate transporters have different functions since they differ in the affinity for ions (Pilsyk and Paszewski, 2009). CrVI uptake by plant roots occurs by active transport, but there is no conclusive information about its translocation mechanisms (Shanker et al., 2009). In contrast to our results, after exposing *Citrullus vulgaris* to different CrVI concentrations, Dube et al. (2003) found that sulfate decreased in the leaves at all Cr levels.

Since CrVI was supplied as $K_2Cr_2O_7$ in this experiment, the beneficial effect of Cr on sulfate uptake was probably from additional K in the solution. K serves as a dominant cation for counterbalancing anions in plants (Marschner, 2012). At higher Cr levels, enhanced Cr uptake in *P. vittata* caused increasing plant K concentrations, probably to balance excessive anions Cr (Fig. 4C). K concentration in the fronds and roots were increased with increasing Cr concentration in the media by 17–39% in the fronds (Fig. 4C).

The addition of sulfate in the growth media containing CrVI did not change sulfur concentration in the fronds and roots of *P. vittata*

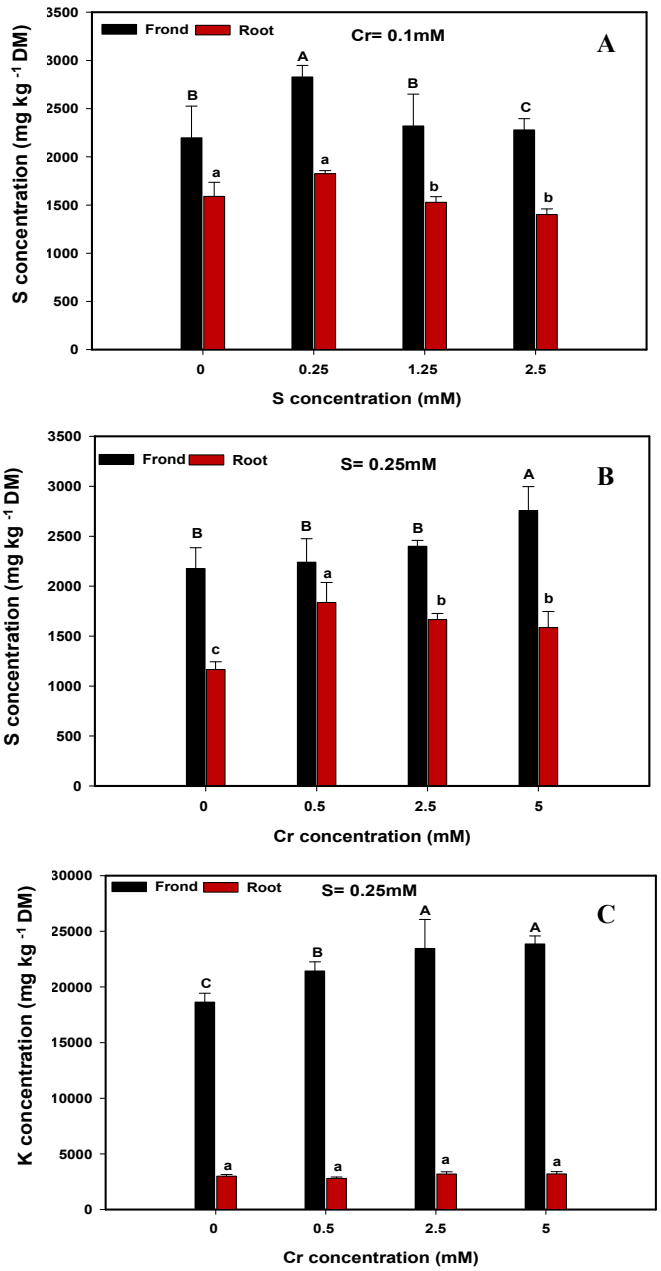

Fig. 4. Effects of CrVI and sulfate on sulfur (AB) or K (C) concentrations in the fronds and roots of *P. vittata* after growing for 1 d in 0.2-strength Hoagland solution containing 0.1 mM CrVI and 0, 0.25, 1.25 or 2.5 mM sulfate (A) and 0.25 mM S and 0, 0.5, 2.5 or 5.0 mM CrVI (B and C). The bars are standard error of the means of three replicates. Treatments followed by the same letters are not significantly different at $\alpha = 0.05$.

(Fig. 4A). For example, as sulfate concentrations in the media increased from 0.25 to 2.5 mM, the sulfur concentrations increased from 2197 to 2278 mg kg^{-1} in the fronds, but decreased from 1589 to 1400 mg kg^{-1} in the roots. The results indicated that the addition of Cr probably induced stress in *P. vittata*, limiting sulfate uptake. Due to its structural similarity with essential nutrient sulfate, CrVI affects plant nutrition by interfering its uptake and translocation (Shiavon et al., 2007, 2012). In the case of *P. vittata*, our results showed that CrVI increased sulfate uptake and translocation at all CrVI levels (Fig. 4B). Similar effect was observed in *P. vittata* with arsenate and sulfate where sulfate uptake in *P. vittata* roots was increased with increasing As concentration in hydroponics (Wei et al., 2010). They found that 15–30 mg L^{-1} of sulfate enhanced *P. vittata* As uptake by 18–85%. In the present study, CrVI and sulfate helped each other in uptake and translocation by *P. vittata*, suggesting that CrVI probably did not share the same transporters with sulfate in *P. vittata*.

4. Conclusion

Though Cr generally inhibits the growth and development of most non-tolerant plants, our results showed that *P. vittata* tolerated 5.0 mM CrVI (260 mgL^{-1}) in the presence of sulfate. *P. vittata* was effective in taking up Cr, but not effective in translocating Cr to the fronds as most of the Cr was concentrated in the roots. While CrVI was provided in the media, 89–93% and 78–96% CrIII was found in *P. vittata* fronds and roots. Reduction of CrVI to CrIII in plants could be one of its detoxification mechanisms. Addition of sulfate increased Cr accumulation in *P. vittata* roots whereas addition of CrVI increased sulfate uptake by *P. vittata*. These results indicated that *P. vittata* had potential in Cr phytoremediation in contaminated sites but further studies are needed to evaluate this potential. However, the mechanisms of how sulfate and CrVI enhance each other's accumulation in *P. vittata* need further investigation.

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