Interactive effects of As, Cd and Zn on their uptake and oxidative stress in As-hyperaccumulator *Pteris vittata*

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**A B S T R A C T**

The effects of arsenic (As), cadmium (Cd) and zinc (Zn) on each other’s uptake and oxidative stress in As-hyperaccumulator *Pteris vittata* were investigated. *P. vittata* plants were exposed to 50 μM As, Cd and/or Zn for 15 d in 0.2-strength Hoagland solution. When applied alone, *P. vittata* accumulated 185 mg kg⁻¹ As, 164 mg kg⁻¹ Cd and 327 mg kg⁻¹ Zn in the fronds. While Cd and Zn did not impact each other’s uptake, As affected Cd and Zn uptake. Whereas As decreased Zn uptake, Zn affected As speciation in *P. vittata* fronds, with more arsenate (AsV) than arsenite (AsIII) being present. At 50 μM As, 75 μM Zn increased As accumulation in *P. vittata* fronds by 10 folds to 2363 mg kg⁻¹ compared to 50 μM Zn. Although AsV was the predominant As species in all tissues, Cd enhanced AsIII levels in the fronds but increased AsV in the roots. Co-exposure of Cd + Zn elevated oxidative stress basing on thiobarbituric acid reactive substances, H₂O₂ content, Evans blue dye uptake, membrane injury index and reactive oxygen species (ROS) relative to single metal. By lowering Cd and Zn concentrations in *P. vittata* fronds, As reduced the associated stress comparative to Cd or Zn treatment. The results enhance our understanding of the mechanisms underlying the interactions between As, Cd and Zn in As-hyperaccumulator *P. vittata*.

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1. Introduction

Arsenic (As) and cadmium (Cd) are toxic metals, which are often co-present in the environment (Anjum et al., 2017; Ronzan et al., 2017). Both As and Cd are top priority hazardous pollutants based on ATSDR (2015). Improper industrial and agricultural practices have elevated their concentrations in the environment (Zare et al., 2018; Shahid et al., 2018).

Elevated concentrations of As and Cd have been reported in groundwater in many countries. While groundwater in Bangladesh contains up to 9 mg L⁻¹ As (de Oliveira et al., 2018), As concentrations in well waters for drinking in Pakistan are 12–488 μg L⁻¹ (Rasool et al., 2017). High As concentrations at 0.06–2.68 mg L⁻¹ have been reported in the Datong Basin of China (Pi et al., 2015). Similarly, Cd concentrations at 40 μg L⁻¹ have been reported in Pakistan (Azizullah et al., 2011). Unlike As and Cd, zinc (Zn) is an essential micronutrient for plant and human health, however, high Zn concentrations in irrigation water in China have been reported (Hu et al., 2018).

At high concentrations, As, Cd and Zn are all toxic to plants (Van der Ent et al., 2012). For example, As is toxic to *Pteris ensiformis* (a non-hyperaccumulator) at 0.7 mg L⁻¹ (de Oliveira et al., 2018). While Cd and Zn are toxic at 5–30 and 100–400 μg kg⁻¹ in the fronds of sensitive plants, hypertolerant plants accumulate up to 100 μg kg⁻¹ Cd and 3000 μg kg⁻¹ Zn (Van der Ent et al., 2012). For example, *Comphrena clausenii* accumulates up to 287 mg kg⁻¹ Cd
and 5318 mg kg⁻¹ Zn in the shoots after exposing to 3 mM Zn and 0.1 mM Cd for 30 d (Carvalho et al., 2014). Sharma et al. (1999) showed that, at 25–50 μM, Zn negatively affected Cd uptake in Zn hyperaccumulator Silene vulgaris and vice versa. Zinc concentrations in soils at < 125 mg kg⁻¹ are optimal for plant growth (Hosseini and Poorakbar, 2013), with mean Zn concentrations in soils at 20–40 mg kg⁻¹ (Kader et al., 2015).

Much research has focused on As, Cd and Zn uptake in plants (Liu et al., 2018; Chen et al., 2017; Balestri et al., 2014; Shi et al., 2015). Plants take up arsenate (As(V)) via phosphate (P) transporters while arsenite (As(III)) uptake is through aquaglyceroporins (Mendoza-Cózatl et al., 2011; Verbruggen et al., 2009). Cd and Zn are taken up by ZIP transporters and are loaded into the xylem by metal P1B-ATPases such as AtHMA2 and AtHMA4 (Zare et al., 2018). In addition, As and Cd translocation is driven by root plasma membrane transporters (Ishimaru et al., 2010; Mendoza-Cózatl et al., 2008).

Due to their chemical similarity, Cd and Zn compete during plant uptake (Benaková et al., 2017). Cherif et al. (2011) reported that excess Zn levels aggravate Cd oxidative stress, while adequate Zn concentrations reduce Cd toxicity. Vera-Estrella et al. (2017) showed that both Cd and Zn uptake was increased in Nicotiana tabacum after exposure to 75 μM Cd in 0.2X Zn. In general, As, Cd and Zn cause oxidative stress in plants by generating reactive oxygen species (ROS), such as O₂⁻, H₂O₂ and IO₃⁻ (Singh et al., 2011; Balestri et al., 2014). Oxidative damage to membrane lipids increases the production of thiobarbituric acid reactive substance (TBARS), whereas injury to cell membranes may lead to their leakage and subsequently cell death (de Oliveira et al., 2016). Among different ROS, H₂O₂ is of great concern due to its long half-life and high permeability across membranes (Gill and Tuteja, 2010).

Arsenic-hyperaccumulator P. vittata is suitable for phytoremediation of As-contaminated sites due to its fast growth, high biomass production and wide distribution. To our knowledge, little information is available regarding the interactive effects of As, Cd and/or Zn in P. vittata. Studies have examined the effects of As and Cd on P. vittata, but until now little is known about the interactions among As, Cd and/or Zn. In this study, we assessed the interactive effects of As, Cd and/or Zn on the growth and stress in P. vittata. The objectives were to investigate the effects of As, Cd and/or Zn on each other’s uptake in P. vittata, and their impacts on the oxidative stress in P. vittata by assessing TBARS, H₂O₂, membrane damage and electrolyte leakage.

2. Materials and methods

2.1. Experimental setup

One year old P. vittata plants of uniform size with 2–3 fronds were cultivated in a greenhouse. The plants were acclimatized in 0.2-strength Hoagland solution (0.2X HS) at pH 5.7 for 3 weeks. Acclimatized plants were transferred to 1L opaque bottles containing Zn-free 0.2X HS. Plant fresh weight (fw) was measured before experiment, with three replicates being used. The water loss via transpiration was compensated via frequent additions of DI water (Benaková et al., 2017). Due to their chemical similarity, Cd and Zn compete during plant uptake (Benaková et al., 2017). Cherif et al. (2011) reported that excess Zn levels aggravate Cd oxidative stress, while adequate Zn concentrations reduce Cd toxicity. Vera-Estrella et al. (2017) showed that both Cd and Zn uptake was increased in Nicotiana tabacum after exposure to 75 μM Cd in 0.2X Zn. In general, As, Cd and Zn cause oxidative stress in plants by generating reactive oxygen species (ROS), such as O₂⁻, H₂O₂ and IO₃⁻ (Singh et al., 2011; Balestri et al., 2014). Oxidative damage to membrane lipids increases the production of thiobarbituric acid reactive substance (TBARS), whereas injury to cell membranes may lead to their leakage and subsequently cell death (de Oliveira et al., 2016). Among different ROS, H₂O₂ is of great concern due to its long half-life and high permeability across membranes (Gill and Tuteja, 2010).

2.2. As, Cd and Zn uptake and As speciation in P. vittata

After growing for 15 d, medium pH was determined, plant roots were washed with DI water and rinsed with ice cold phosphate buffer (1 mM Na₂HPO₄, 10 mM MES and 0.5 mM Ca(NO₃)₂, pH 5.7) to remove adsorbed As, Cd and Zn. They were then washed again with DI water to remove the buffer (de Oliveira et al., 2018). The roots and fronds were separated and fresh weights were recorded before being oven-dried at 65 °C to constant weight. Fresh samples were saved in liquid nitrogen to obtain homogeneous samples for As speciation and for measurements of plant physiological parameters.

Oven-dried plant samples (-0.1 g) were digested with HNO₃/H₂O₂ on a hot block digester (Environmental Express, Mt. Pleasant, SC) using USEPA Method 3058B (de Oliveira et al., 2017). Digested solutions were filtrated (0.45 μm). The plant digestions and sampled hydroponic solutions were analyzed for total As, Cd and Zn concentrations by inductively-coupled plasma spectrometry (ICP-MS, PerkinElmer 5300DV, Waltham, MA) with USEPA Method 2007. All samples were diluted with 5% HNO₃.

Arsenic speciation in P. vittata was analyzed by ultrasonically extracting fresh plant with 10 mL methanol: water (1:1 v/v) for 4 h at 30 °C two times (Zhao et al., 2015). The extracts were decanted into a 50-mL volumetric flask and diluted to 50 mL with DI water. AsV and AsIII were separated by an As speciation cartridge (Waters SPE cartridge) with AsV being retained (Mathews et al., 2010) and AsIII was determined by ICP-MS. Arsenic speciation in the growth solution after growing P. vittata was separated using the cartridge and determined with ICP-MS. In addition, standard plant reference material 1547 (tomato leaves) from the National Institute of Science and Technology (Gaithersburg, MD) and appropriate reagent blanks, internal As standard at 100 μg L⁻¹ were measured every 20 samples to monitor the stability of ICP-MS. The samples were spiked to a final concentration of 20 μg L⁻¹ to ensure the accuracy and precision of ICP-MS.

2.3. As, Cd and/or Zn-induced oxidative stress in P. vittata

Stress assays including TBARS and H₂O₂ were used to examine the oxidative damage induced to P. vittata fronds and roots after exposing to 50 μM As, Cd and/or Zn for 15 d. The TBARS content was determined following de Oliveira et al. (2016) by quantifying lipid peroxidation in P. vittata fronds. Briefly, -0.3 g of fresh-tissue was mixed with 1.5 mL of 5% (wt/v) trichloroacetic acid (TCA) solution in an ice bath. The homogenate was transferred into fresh tubes and centrifuged at 10,000 g for 10 min at room temperature. To 1 mL supernatant, 1 mL of 20% (w/v) TCA containing 0.5% (w/v) TBA was added. The mixture was heated at 95 °C for 30 min, then quickly cooled on ice, with the absorbance being measured at 532 and 600 nm (Shimadzu UV1600, Columbia, USA). The TBARS content was calculated as μmol g⁻¹ using an extinction coefficient of 155 μM⁻¹ cm⁻¹.

The H₂O₂ content was determined according to Junglee et al. (2014). Briefly, -0.1 g fresh frond tissue was ground to homogenized powders using liquid nitrogen. In two samples, 1 mL control or test homogenization solution was added respectively before grinding without light. It was centrifuged at 12,000 g, 4 °C for 10 min, with absorbance being determined spectrophotometrically at 250 nm. The control value was deducted for background correction. Tissue H₂O₂ concentration was derived from a standard curve based on 0, 0.1, 0.2, 0.5 and 1 mM H₂O₂ via linear regression.
To determine the impacts of As50, Cd50 and/or Zn50 on the membrane stability of P. vittata roots, Evans Blue dye uptake test was performed (Das et al., 2017). The stain cannot penetrate the membrane in living cells, thereby indicating the loss of root plasma membrane integrity when visible cytoplasm stains appear. Briefly, the roots were stained with 0.025% (w/v) Evans Blue solution in 100 mM CaCl2, pH 5.6 for 10 min. Then they were washed with 100 mM CaCl2, homogenized with 1% (w/v) sodium dodecyl sulfate, centrifuged at 13,500 × g for 10 min, and recorded the absorbance at 600 nm. Root and frond membrane integrity was determined by recording leachate electrical conductivity in DI water at 40 °C and 100 °C (Das et al., 2017; Almeselmani et al., 2006), with the injury index being calculated as C40/C100 or C20/C14 (Das et al., 2017; Almeselmani et al., 2006), with the injury index based on membrane injury index was determined by recording leachate electrical conductivity in DI water at 40 °C and 100 °C (Das et al., 2017; Almeselmani et al., 2006), with the injury index being calculated as C40/C100 

2.4. Data analysis

Data are presented as the mean of three replicates with error bars representing standard error. Significant differences were determined by two way analysis of variance and treatment means were compared by Duncan’s multiple range test at p < 0.05 with statistical software (SAS Version 13.1 Inc., Cary, NC, USA).

3. Results and discussion

To determine the interactive impacts of As, Cd, and/or Zn on P. vittata growth and their uptake, plants were exposed to 50 μM As, Cd and/or Zn (As50, Cd50, and/or Zn50) for 15 d. In addition, As speciation in plant tissues and growth media, and plant oxidative stress based on TBARS, H2O2, Invitrogen ROS, and root and frond membrane damage were determined.

3.1. Plant biomass and metal accumulation in P. vittata

Plant biomass reflects the impact of metals on its growth. All treatments showed no significant effect on P. vittata biomass, with roots and fronds being 0.49–0.61 and 0.56–0.81 g plant⁻¹ dw, respectively (Fig. 1a). In addition to biomass, metal concentrations in plants were also determined. After exposing to As50, Cd50, or Zn50, the As, Cd, and Zn concentrations were 153, 4738 and 719 mg kg⁻¹ in the roots, and 185, 164 and 327 mg kg⁻¹ in the fronds, respectively, with 3–29 folds more Zn and Cd being accumulated in the roots (Fig. 1b).

Under co-exposure, As significantly (p ≤ 0.05) reduced the Cd and Zn concentration in P. vittata roots, while As concentration was not impacted by Cd or Zn (Fig. 1a). For example, compared As₁₀₀+Cd₅₀ to Cd₅₀ treatment, As decreased root Cd (4738 to 817 mg kg⁻¹) and frond Cd (164 to 88 mg kg⁻¹) concentrations without As concentration being impacted (Fig. 1b). The results were different from Ronzan et al. (2017) who reported that, compared As₁₀₀+Zn₅₀ to As₁₀₀ treatment, Cd reduced the As concentrations in P. vittata fronds from 460 to 400 mg kg⁻¹ after 15 d exposure in a hydroponic study.

However, similar findings were reported by Ghiani et al. (2014) in plant Trifolium repens where lower Cd concentrations in the roots and shoots were observed in As₂₀₀+Cd₅₀ than Cd₅₀ treatment after 15 d exposure in soil. Similar to Cd, when comparing As₅₀+Zn₅₀ to Zn₅₀ treatment, As also significantly (p ≤ 0.05) decreased root Zn (1018 to 396 mg kg⁻¹) and frond Zn (327 to 65 mg kg⁻¹) concentrations without As concentration being impacted (Fig. 1c).
concentrations were not impacted by As after 12 week exposure. It is known that As uptake mechanism is different from Cd and Zn (Zare et al., 2018; Mendoza-Cózatl et al., 2011), but it was unclear why As reduced plant Cd and Zn concentrations while Cd and Zn showed little impact on As uptake.

In addition, Cd and Zn had little impact on each other’s uptake in P. vittata. Similar to Cd50 or Zn50 treatment, higher Cd (2709 vs. 345 mg kg\(^{-1}\)) and Zn (1377 vs. 345 mg kg\(^{-1}\)) concentration was in the roots than fronds under Cd50+Zn50 treatment (Fig. 1bc). This was consistent with Benaková et al. (2017) who reported higher Cd and Zn concentrations in the roots than shoots in Brassica napus after exposing to Cd5+Zn10 for 14 d, which was typical of non-hyperaccumulators (Zare et al., 2018). However, it was interesting that P. vittata took up more Cd than Zn in the roots after exposing to 50 μM Cd or Zn (4738 vs. 1018 mg kg\(^{-1}\)). This is similar to Arabidopsis halleri, which showed higher Cd uptake than Zn in the field as it extracted 23% Cd and 3.0% Zn from the soil after 9-month growth (Zhang et al., 2017). In comparison, P. vittata concentrated 3 and 29-fold Zn and Cd more in the roots than shoots (Fig. 1bc). By keeping more Cd and Zn in the roots, they would have limited impact on various metabolic activities in P. vittata fronds.

3.2. Arsenic reduced Cd– and Zn-induced oxidative stress in P. vittata

Among all treatments, only under co-exposure with Cd50+Zn50 were phytotoxicity symptoms observed in P. vittata fronds including necrosis and chlorosis (data not shown). This may be attributed to their combined toxicity, which was consistent with their concentrations in P. vittata (Fig. 1bc).

Metal accumulation in plants induces oxidative stresses, causing production of TBARS in tissues (Chen et al., 2018; Shahid et al., 2017). Among the three metals, As caused the lowest TBARS, with little difference between Cd and Zn (Fig. 2a). Though Zn and Cd concentrations were greater in the roots (1018 and 4738 mg kg\(^{-1}\)) than those in the fronds (327 and 164 mg kg\(^{-1}\)), the TBARS were lower in the roots than those in the fronds. Compared to Cd50 treatment, Cd50+As50 increased TBARS content by 60–80% (p < 0.05), while compared to Zn50 treatment, Zn50+As50 reduced TBARS by 56–75% (Fig. 2a). Our data showed that As reduced both Zn (63–80%) and Cd (46–83%) accumulation in P. vittata (Fig. 1bc), which was inconsistent with TBARS data. For example, As reduced the Cd concentrations in P. vittata roots and fronds from 4738 to 817 and 164 to 88 mg kg\(^{-1}\) relative to Cd50 treatment (Fig. 1bc). However, compared to Cd50, increased TBARS were observed in P. vittata roots (60–108 μmol kg\(^{-1}\)) and fronds (79–128 μmol kg\(^{-1}\)) under As50+Cd50. It was possible that As accumulated in P. vittata contributed to oxidative stress besides Cd (Fig. 1a).

In addition to TBARS, the H2O2 contents in P. vittata after exposing to As50 or Cd50 and/or Zn50 were examined (Fig. 2b). As an important ROS, H2O2 is produced by SOD-catalyzed dismutation of superoxide radical (O\(_2^−\)), which is generated during electron transport in photosynthesis and respiration (Shahid et al., 2017; Gill and Tuteja, 2010), thereby reflecting oxidative stress levels (Das et al., 2017). The H2O2 in P. vittata was increased compared to the control, indicating metal-induced stress. Compared to Cd50 treatment, Cd50+As50 increased H2O2 content by 50% in the fronds (p < 0.05), while compared to Zn50 treatment, Zn50+As50 reduced H2O2 content by 50% in the roots (Fig. 2b). The trend for TBARS and H2O2 was consistent, both showing lower stress under Zn50+As50 than Zn50 treatment and higher stress under Cd50+As50 than Cd50 treatment. Liu et al. (2007) also reported similar findings in plant T. aestivum as more oxidative stress was noticed under combined exposure of As + Cd compared to Cd treatment. When As and Cd co-present, they induced more stress than single metal treatment.

addition, to our knowledge, there is no hydroponic study available for combined stress of Zn + As as only one study on Cucumis sativa regarding combined stress of Zn + As in soil was examined (Kader et al., 2015). Both TBARS and H2O2 levels were determined in plant roots to study abiotic and biotic stresses caused by heavy metals (de Oliveira et al., 2018; Das et al., 2017). TBARS are used to assess the extent of membrane damage by lipid peroxidation and deterioration of membrane integrity. Increase in the amount of lipid peroxidation under environmental stresses are paralleled with increased production of ROS (Sharma et al., 2012). Therefore, the level of lipid peroxidation has been widely used as an indicator of ROS-mediated damage to cell membranes under stressful conditions (Shahid et al., 2015). Enhanced lipid peroxidation takes place when ROS level reaches above threshold as lipid peroxidation augments the oxidative stress through production of lipid-derived radicals that themselves can react with and damage proteins and DNA (Shahid et al., 2013). However, follow up experiment is needed to understand why this was the case. The oxidative stress in...
P. vittata was increased under Cd50+Zn50 treatment than Cd50 or Zn50 treatment alone, with TBARS and H2O2 contents being greater in the fronds than the roots, which was opposite to their concentrations.

In addition to TBARS and H2O2 contents, we also determined the localization of H2O2 in P. vittata root tips after exposure to As50, Cd50 and/or Zn50 alone (Fig. 2c). This is because ROS localization inside root cells plays an important role in cell division and differentiation (Yu et al., 2016). It is expected that low H2O2 in the roots helps to mitigate stress, which was observed in the negative control plants. However, in the roots of positive control incubated with 1% H2O2 for 1 h, the staining intensity was the highest (Fig. 2c). As expected, more H2O2 was observed under Cd50+Zn50 stress, consistent with the TBARS and H2O2 results (Fig. 2bc). It has been reported that at low concentrations, H2O2 acts as a signal molecule involved in triggering tolerance to various stress (Shahid et al., 2017). In plants, appropriate H2O2 levels promote expression of defense and resistance genes (Quan et al., 2008). Further, plant roots are the first in contact with contaminants, so they are sensitive to metal stress (Huang et al., 2012). It is known that metal-induced ROS is common in plants, which acts as secondary messengers regulating cell growth and differentiation (Zanella et al., 2016; Tsukagoshi et al., 2010).

3.3. Membrane integrity and stability under metal stresses in P. vittata

Evans Blue dye uptake and membrane injury index were performed to assess the impact of As50, Cd50 and/or Zn50 treatment on the membrane stability of P. vittata (Fig. 3). The Evans Blue assay is based on dye accumulation inside dead cells, while the membrane injury index assay is based on electrolyte leakage from dead cells, with ion leakage as a marker of cell death (Das et al., 2017; Rolny et al., 2011). The Evans Blue assay showed that all three metals decreased the membrane stability of P. vittata compared to the control, with Zn and As reducing each other's impact, and Zn and Cd increasing each other's impact (Fig. 3a). Under Cd50+Zn50 treatment, extensive cell death was induced in roots cells, with OD600 increased to 0.35 from 0.02 in the control (Fig. 3a). Less cell death occurred under As50+Zn50 treatment with OD600 at 0.11 compared to Zn50 at 0.19. The data were consistent with the fact that As reduced Zn uptake (Fig. 1bc) and lowered the TBARS and H2O2 content in P. vittata (Fig. 2ab).

Similar to membrane injury data, all three metals increased the electrolyte leakage of cells, with Cd and Zn showing greater impact than As, and with Zn and As reducing each other's impact (Fig. 3bc). The membrane injury index in control cells were 15–29%, which increased to the highest at 73–94% under Cd50+Zn50 treatment. Among different metals, the frond membrane injury index data were consistent with the TBARS and H2O2 data, showing greatest membrane damage occurred under Cd50+Zn50 (Fig. 2ab).

In this study, more Zn and Cd were accumulated in the roots (1038 and 4738 mg kg\(^{-1}\)) than the fronds (327 and 164 mg kg\(^{-1}\)) in P. vittata (Fig. 1bc). However, more oxidative stress was observed in the fronds than the roots based on TBARS, H2O2, and root and frond membrane damage (Figs. 2 and 3). It is possible that root tissues are more stress-resistant due to their robust anatomy, extensive proliferation, lignin deposition in the cells walls and active enzymatic activity (Mahmood et al., 2016). In contrast, plant shoot tissues may be more prone to damages as more cytoplasmic organelles (mitochondria and chloroplast) and biological membranes are present in the shoots than roots (Roccotello et al., 2010). However, more oxidative stress was shown in the fronds than roots, and the mechanisms of how As impacted Cd-and Zn-induced oxidative stress deserve further study.

3.4. Impact of Cd and Zn on As speciation in P. vittata

In addition to monitoring metal concentrations, the impacts of Zn and Cd on As speciation in P. vittata were also determined. Both AsV and AsIII were predominant species in P. vittata (Fig. 4). For a better comparison, AsV and AsIII concentrations in the growth media were also determined, showing ~100% as AsV (Fig. 4ab). In addition, Cd50 had no impact on As speciation, with more AsV in the roots and AsIII in the fronds, typical of As-hyperaccumulators (Ma et al., 2001). However, Zn50 significantly decreased As reduction from AsV to AsIII in P. vittata, thereby increasing the frond and root AsV by 9- and 16-fold (Fig. 4a).

In P. vittata fronds, AsIII was predominant under As50 or As50+Cd50 exposure. AsV is reduced to AsIII by AsV reductase in plants, which depends on glutaredoxin, a glutathione dependent thiol transferase (Kertulis-Tartar et al., 2009). The AsV reductase activities were enhanced in P. vittata under As stress, as it is more effective for P. vittata to translocate AsIII to the fronds (de Oliveira et al., 2014). Further, Lei et al. (2012) reported that AsIII was...
As and Zn concentration in the roots (c) and in P. vittata fronds (d). Bars are ±SE of the means of three replicates. Treatments followed by same letters are not significantly different at p ≤ 0.05.

subsequently complexed with phytochelatins, leading to AsIII storage in the vacuoles. However, under As50+Zn75 treatment, 90% of the As in P. vittata fronds was AsV (27 vs. 3.0 mg kg⁻¹; Fig. 4a). The data suggested that Zn decreased AsV reductase activity in P. vittata tissues.

To further confirm the effect of Zn on As speciation, P. vittata was grown in 0.2X HS for 15 d under 25 or 75 μM Zn (Fig. 4b). At lower Zn concentration (As50+Zn25), 91% AsIII was present in the fronds (742 vs. 76 mg kg⁻¹) and 61% AsV in the roots (25 vs. 16 mg kg⁻¹), typical of P. vittata. However, at higher Zn concentration (As50+Zn75), AsV dominated in both fronds (82%; 1944 vs. 419 mg kg⁻¹) and roots (91%; 200 vs. 19 mg kg⁻¹). The data showed that, with increased Zn concentrations from 25 to 75 μM, substantially more AsV was present in P. vittata fronds and roots. In addition, compared to As50+Zn25, the As concentrations in the fronds (818–2363 mg kg⁻¹) and roots (41–219 mg kg⁻¹) in P. vittata under As50+Zn75 were increased by 3–5 folds (Fig. 4d). Why Zn decreased AsV reduction to AsIII and increased As concentrations in P. vittata warrants more examination.

In addition to As, the root Zn concentration was 7-fold higher under As50+Zn75 treatment than that at As50+Zn25 (2375 vs. 349 mg kg⁻¹) (Fig. 4c). The Zn concentration in the fronds under As50+Zn75 was 2-fold that of As50+Zn25 (2171 vs. 924 mg kg⁻¹), in addition to displaying a 3-fold increase in As concentration (2363 mg kg⁻¹; Fig. 4d). The findings suggested that Zn affected As speciation in P. vittata, with much greater As uptake and greater AsV than AsIII being observed with increasing Zn concentrations.

4. Conclusion

In this study, the impacts of As50, Cd50 and/or Zn50 on the biomass, metal uptake and stress in P. vittata were investigated. The data showed that Zn increased As uptake at As50+Zn75 treatment while lower As concentration was observed in the fronds with As50+Cd50 than Cd50 treatment. Although AsV was predominant in the roots and AsIII in the fronds, the presence of Zn75 enhanced AsV levels in both fronds and roots. In addition, based on TBARS and H2O2 contents in P. vittata, lower oxidative stress was observed in As50+Zn50 than Zn50 treatment, with the highest stress occurring in Cd50+Zn50 treatment. The inhibition of Zn on AsV reduction to AsIII in P. vittata was reported for the first time. Although, the mechanisms underlying the interactive effects of As, Cd and/or Zn in plants are yet clear, this study showed that Zn not only decreased reduction of AsV to AsIII, but also increased As uptake by P. vittata. Such information can be used to enhance As uptake by P. vittata to increase its efficiency in phytoremediation of As-contaminated soils.

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