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RESEARCH ARTICLE

Phosphate Amendments Moderate the Arsenate Accumulation and Its Subsequent Oxidative and Physiological Toxicities in *Amaranthus viridis* L.

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Abstract Arsenic, a carcinogenic metalloid, may enter into food chain through the consumption of crops irrigated with arsenic contaminated underground water. The traditional prevention of the arsenic entry into the food chain in a vast scale of agriculture is very difficult. The only way to prevent this entry is to increase the supply of arsenic analogue phosphate, an essential plant growth nutrient in soil. Two-factor randomized hydroponic experiment (3 arsenate × 5 phosphate concentrations) was performed for 28 days with 11 days old seedlings of *Amaranthus viridis* L. Concentrations of superoxide dismutase, catalase, peroxidase and malondialdehyde increased up to 2.12, 1.47, 1.86 and 3.66 folds, respectively at concentration of 40 μM arsenate. Addition of phosphate resulted in the decrease in arsenic accumulation by 68.18 %. At 400 μM phosphate amendment, there were also a decrease in the levels of superoxide dismutase, catalase, peroxidase and malondialdehyde up to 21.52, 26.55, 28.44 and 37.15 %, respectively. The toxicity reduction at physiological level was observed as nearly 1.8 fold increase in the contents of both chlorophyll and biomass. The arsenic entry into food chain via plants can thus be prevented by the addition of phosphate into the growth medium.

Keywords *Amaranthus viridis* L. · Antioxidant enzymes · Arsenate · Moderate · Phosphate · Toxicity

Introduction

The entry of any heavy metal/metalloid causes oxidative and physiological toxicity in the plants [1–4]. The oxidative toxicity results due to the generation of reactive oxygen species (ROS) [1]. Since ROS is toxic at the cellular level, plants inherit some mechanisms to counteract this stress. The mechanisms involve the generation of metal binding thiol (SH) rich phytochelatins and antioxidants [4, 5]. The antioxidant dependent ROS scavenging comprises the non enzymatic antioxidants, mainly involving the glutahione (GSH), ascorbate and carotenoids, as well as the enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) etc. [1]. The generated ROS is acted upon by the SOD, which catalyzes the disproportionation of superoxide anion (O_2^-) to H_2O_2 and O_2. CAT and POD further convert the in situ produced H_2O_2 to harmless products like H_2O as well as some phenolic compounds [4, 6]. Other than the elevation of antioxidant level, ROS also causes damage to the cell membrane. The damage involves the peroxidation of membrane lipids, generating malondialdehyde (MDA). Thus, by estimating the concentration of antioxidant enzymes and MDA in the plant tissues, the level of oxidative damage can be determined. At the physiological level, metal toxicity in plants affect the growth, photosynthesis, biomass, nitrogen and phosphorus storages etc. [2].

Arsenic (As), a ubiquitous metalloid of environment, occurs in various oxidation states as +5 (arsenate), +3 (arsenite), 0 (elemental) and −3 (arsenide). Although arsenite is the most toxic form, in the aerobic environment

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arsenate is the predominant species [7]. The metalloid occurs geogenically, as arsenopyrite ($FeAsS_2$) [8, 9]. The anthropogenic contaminations include mining activities, applications of arsenical herbicides and insecticides as well as irrigation. The soil—As then enters the food chain via primary consumers, and finally, reaching the humans to cause the health hazards [8]. To prevent the possible entry of As into the food chain, it will be advantageous to employ any naturally occurring mechanism that could moderate the uptake and accumulation of As in food crops growing in the contaminated soil. Physiological and electrophysiological studies have revealed that arsenate and phosphate share common transport pathway via roots of the higher plants [10]. Since, phosphate is a growth supporting nutrient, plants prefer its uptake over arsenate [11–14].

With the understanding of the above molecular analogy between arsenate and phosphate, the most cost effective method for amelioration of arsenic toxicities, at the oxidative and physiological levels, in agriculture would be the prevention of its entry in the food chain using phosphate amendments. Thus, this study has been designed to determine the effect of arsenate-phosphate amendments on the variation in the physiological and oxidative toxicities in the root and shoot tissues of *Amaranthus viridis* L. Since, *A. viridis* is consumed widely in different provinces of India and has a short growth cycle as well as left uninvestigated as per similar study, it is considered for the present work. The hydroponic experiment involves the plant cultivation under combination of added arsenate (as $Na_2HAsO_4.7H_2O$) and phosphate (as NaH_2PO_4) supplies in a factorially designed (3 × 5) pattern for the period of 28 days. The root and shoot tissues were subjected to the determination of arsenic accumulation, physiological parameters (viz. chlorophyll, biomass, phosphorus and nitrogen accumulations), antioxidative parameters (viz. SOD, CAT, POD) and MDA content.

Material and Methods

Experimental Design

Effects of various phosphate (PO_4^{3-}) amendments on arsenate (AsO_4^{3-}) toxicity in *A. viridis* L. were assessed by performing two-factor (in 3 × 5 pattern) complete randomized experiment, in which, the plants were supplied with three concentrations (10, 20, 40 μM) of AsO_4^{3-} (as $Na_2HAsO_4.7H_2O$; Merck, India), each amended with five concentrations (50, 100, 200, 300, 400 μM) of PO_4^{3-} (as NaH_2PO_4; Merck India). There were two sets of control. The first set (0 μM AsO_4^{3-} with 400 μM PO_4^{3-}) was compared with the AsO_4^{3-} supplied plants without PO_4^{3-} amendment, whereas the second set (10–40 μM AsO_4^{3-} with 0 μM PO_4^{3-}) was compared with the respective level of AsO_4^{3-} supplied plants amended with five concentrations of PO_4^{3-}.

For germination, seeds of *A. viridis* were wrapped in sterilized tissue paper towels in half strength nutrient solution [12] and incubated at 25 °C for 72 h. Meanwhile, refined sand was prepared for the pot experiment and a thorough washing with 3 % HCl was done to leach out all of the adsorbed minerals. This was followed by soaking the sand in distilled water for 5 h, and then washing with distilled water at least three times to maintain pH 7.0, followed by air drying and autoclaving. Four kilograms of the treated sand was filled in each plastic pot and soaked with 2 L full strength nutrient solution. In the green house, the 3 days old seedlings were planted at the density of 5 individuals pot^{-1} and grown for 10 days at 25 ± 5 °C under natural light and dark conditions. The volume of nutrient solution was maintained every 3 days. At the 11th day, the tender seedlings were thinned at the density 1 pot^{-1}, followed by the AsO_4^{3-} and PO_4^{3-} treatments. Fifteen pots were supplied with AsO_4^{3-} and PO_4^{3-} as per above mentioned (3 × 5) fashion. All together with the controls, total number of treatment pots obtained were nineteen. Since, the treatments were performed in three replicates, final number of pots in the experiment was 57. This experiment was conducted for 28 days, with the 11 days old plantlets.

A similar experiment with 57 pots as above was conducted with 11 days old plantlet density of 5 pot^{-1} and the variation of antioxidant enzyme levels was measured on the first day of the AsO_4^{3-}–PO_4^{3-} treatments at the interval of 0, 1, 3, 6 and 12 h.

Plant Harvest and Determination of Chlorophyll and Biomass

After 20 days, the AsO_4^{3-}–PO_4^{3-} treated plants were harvested and each separated into root and shoot parts. Chlorophyll a and b were measured in the fresh leaves of *A. viridis* by the standard extraction method using 4 + 1 acetone:ethanol as extraction solvent, measuring the optical density at 663 and 665 and substituting the OD values in standard formulae [15].

Loosely adhered sand particles from the roots were removed by gently washing them under flowing tap water, followed by 3 % HCl rinse, to leach off the adsorbed minerals. The shoot and acid rinsed roots of individual plants were washed with double distilled water at least three times before transferring in a drought oven set at 80 ± 5 °C for incubation till the achievement of constant dry weight (DW). The DW biomass of plants (shoot + root) was measured using electronic balance (Oriental Sales, India) and expressed as g $plant^{-1}$ DW.

Digestion Method and Determination of Total As, P and N in Plant Material

The dried root and shoot samples were utilized for the estimation of As, P and N. Prior to estimation, the dried plant material was grinded (<200 μm size) using a stainless steel grinder (Philips, India). For the spectrophotometric determination of As [16], the ground-materials were digested using 2 + 1 HNO_3:$HClO_4$ [17]. The absorbance (at 644 nm) of samples obtained was plotted on the standard graph to estimate the amount of total As in μg g^{-1} DW.

For the analysis of total P and N, the ground material (25 mg each) was digested using the standard procedure of Langner and Hendrix [18]. For the estimation of P in the digests, standard $SnCl_2$ method was used [19], while, N was determined by the standard phenol disulphonic acid method [20]. The ODs at 690 and 410 nm were calibrated in the standard graphs for the estimation of total P and N, respectively.

Extraction and Estimation of Antioxidant Enzymes and Lipid Peroxidation in Plant Material

One individual of *A. viridis* from each pot of the second set of experiment was harvested at the intervals of 0, 1, 3, 6 and 12 h from the start of As–P treatments and utilized for the analysis of antioxidant enzyme activities and lipid peroxidation. The enzymes were extracted from frozen tissues by adding 0.1 M Tris–HCl buffer, 1 mM EDTA, 4 % polyvinyl pyrrolidone and 1 mM dithiotreitol [21].

Superoxide dismutase (SOD) (EC 1.15.1.1) and catalase (EC 1.11.1.6) activities in the enzyme extracts were determined using standard procedures [22, 23]. A modified procedure [24, 25] was used for the determination of peroxidase (POD) (EC 1.11.1.7), in which the reaction mixture was prepared by mixing 100 mM sodium phosphate buffer (pH 6.0), 30 % H_2O_2 and guaiacol. One milliliter of enzyme extract was added to 3 mL reaction mixture to start the reaction and the increase in OD_{470} at every 30 s was recorded for a period of 2 min. The activity was denoted by U mg^{-1} (1 U denotes 0.1 changes in OD_{470}).

For measuring lipid peroxidation in root and shoot tissues, malondialdehyde (MDA) levels were determined. MDA was extracted from 0.5 g each of root and shoot tissue by homogenizing in 10 % trichloroacetic acid followed by centrifugation at 4000×*g* [21]. The MDA (μM g^{-1} DW) in the supernatant was determined by the standard procedure of Beuge and Aust [26].

Statistical Analyses

The data in the figures are mean ± standard error of three replicates of the treatments. The significance of differences ($p < 0.05$, 0.01 and 0.001) between control and the treatments was calculated by Student's *t* test for paired samples. Correlation statistics (as r-values) were expressed as scattered plots as well as tables, whichever applicable. The softwares used for the above statistics were Statistica v5.52.164.0, Microsoft Office Excel 2003, 2007.

Results and Discussion

The present study has been designed in a 3 × 5 complete randomized factorial fashion to understand the role of phosphate (PO_4^{3-}) amendment in moderating the arsenate (AsO_4^{3-}) toxicity in the leafy vegetable *Amaranthus viridis* L. The variation in toxicity has been determined by assessing the levels of physiological parameters like total chlorophyll, biomass, total-P and N contents; as well as antioxidant enzymes and degree of lipid peroxidation. In general, AsO_4^{3-} toxicity increased in a dose-dependent manner, whereas, the PO_4^{3-} amendments in the medium caused a significant moderation of the toxicity, as determined by the above parameters, when compared with respective controls.

The accumulation of total-As in root and shoot tissues of *A. viridis* (Fig. 1a, b) increased by 1.6 and 1.8 folds, respectively in dose dependent manner (from 10–40 μM AsO_4^{3-} supplies). In comparison to the present study, As accumulation in *Hydrilla* (568.3 μg g^{-1} DW) was greater [27]. By differentiating the total-As accumulation at the tissue level, it was found in the present study that the roots of *A. viridis* accumulated up to twofold higher amount of total-As than that in shoot (Fig. 1a, b). This might be because the toxic metal is immobilized in the vacuoles of the root cells, so that the aerial parts, performing essential metabolic processes, get less affected [14]. In contrast, the hyperaccumulator Chinese brake ferns accumulated higher amount of total-As in the aerial than the underground parts [28, 29], reflecting their phytoextraction potential. When the PO_4^{3-} was amended along with the AsO_4^{3-} supplies, the total-As accumulated in the root and shoot tissues was significantly ($p < 0.001$) reduced by the maximum of 68.18 and 64 %, respectively, with respect to control (0 μM PO_4^{3-} amendment). These observations were further supported statistically by obtaining significant ($p < 0.05$) negative correlation coefficient values between the total As accumulation versus total P stored in root and shoot tissues (Fig. 1c). Except chickpea, the present study was consistent with Chinese brake fern, water fern and *Hydrilla* regarding the antagonistic interaction between PO_4^{3-} and AsO_4^{3-} during their uptake process [2, 3, 14, 27, 28]. Additionally, *A. viridis* accumulated more total-As (up to 226.42 ± 8.42 μg g^{-1}) than chickpea (40 μg g^{-1}) under the influence of PO_4^{3-} amendments. Owing to the

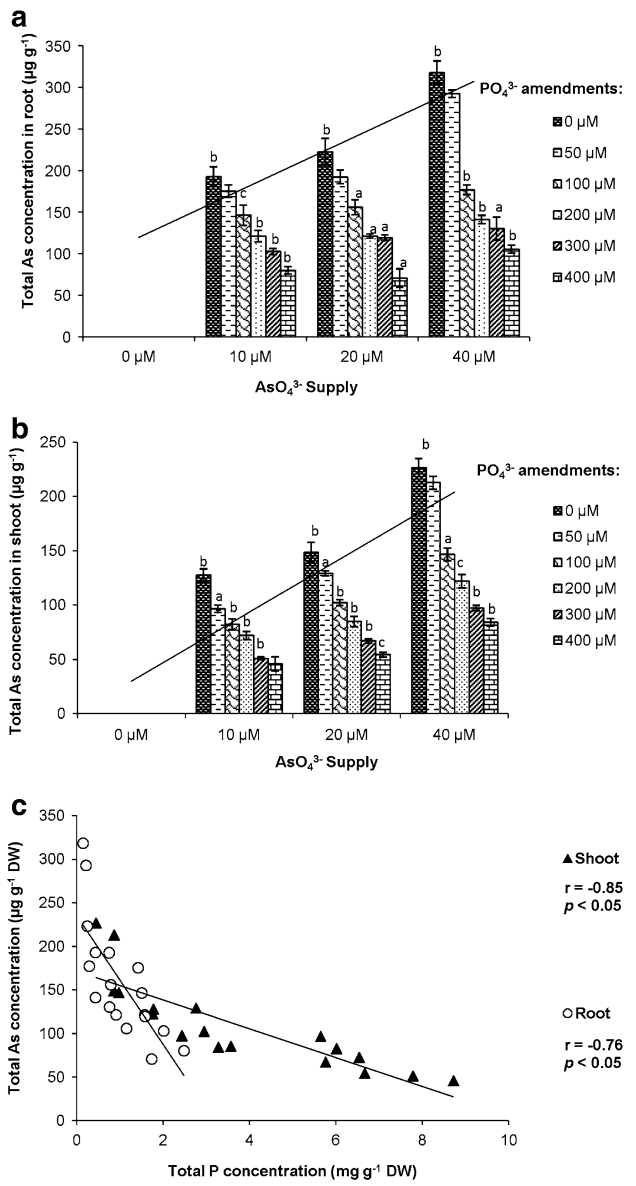

Fig. 1 **a** Variations of total-As accumulation (µg g^{-1} DW) in root and **b** shoot tissues of *A. viridis* with various AsO_4^{3-}/PO_4^{3-} amendments at 28 days of experiment. *Error bars* represent SE of means (n = 3). The *alphabets a*, *b* and *c* indicate values that differ significantly from control at $p < 0.05$; $p < 0.01$ and $p < 0.001$, respectively. *Columns without any of the alphabets* represent differently significant results; **c** relationship between total-As and P accumulations in root and shoot tissues of *A. viridis,* each with representations of Pearson's correlation coefficient (r-value) and level of significance (*p* value)

moderating effects of PO_4^{3-} in *P. vittata,* the total-As accumulation was reduced by 76 and 46 % in root and fronds, respectively, when the concentration of PO_4^{3-} was increased from 20 to 100 µM at 80 µM AsO_4^{3-}. Being a non essential ion for plants, AsO_4^{3-} shares the protein-transporters of its chemical analogue-PO_4^{3-} for the entry via roots, thus reflecting its competitive behavior towards PO_4^{3-} [2, 10, 27]. Hence, the competitive nature of transport might be responsible for the inverse relationship between PO_4^{3-} and AsO_4^{3-} uptake and accumulation in root and shoot tissues of *A. viridis*. Furthermore, since, PO_4^{3-} is a growth supporting macronutrient and AsO_4^{3-} imposes toxicity, plants prefer the uptake of PO_4^{3-}, over AsO_4^{3-} [1, 12, 28].

Photosynthesis is an essential physiological process occurring in phototrophs and is responsible for the conversion of solar energy to chemical energy, hence, acting as a major deciding factor for the formation of plant biomass [27, 30–32]. It is a well understood fact that any type of abiotic stress (including heavy metal), severely affects these physiological parameters [33]. Thus, in the present study, the photosynthetic pigments, namely chlorophyll a and b, as well as biomass were estimated to confirm the AsO_4^{3-} toxicity at the physiological level, and its further moderation due to PO_4^{3-} amendment. The levels of total chlorophyll (Fig. 2a, b) and biomass (Fig. 2c, d) gradually declined (total chlorophyll: 85.36 %; biomass: 88.08 and 67.05 % for root and shoot, respectively) with the respective increase in concentration of AsO_4^{3-} supplies without PO_4^{3-} amendments. Significant ($p < 0.05$) inverse relationships between total-As accumulation and chlorophyll (Fig. 3a)/biomass (Fig. 3b) further confirmed the toxic effects of AsO_4^{3-} supplies on the two physiological parameters. Various plants, like clover, *Hydrilla*, rice, duckweed [1, 27, 30, 31] etc., have confirmed the toxic impacts of AsO_4^{3-} on the above parameters. The chlorophyll a + b reduction in *A. viridis* was remarkably high (up to 85.36 %) as compared to clover (12 % reduction), *Hydrilla* (25.77 % reduction) and rice (60.54 % reduction). Comparing the biomasses of Chinese brake fern with the *A. viridis,* the biomass of the former was less affected (reduced up to 51 and 40 % in roots and fronds, respectively) by AsO_4^{3-} toxicity [29]. The reason behind this difference might be due to the fact that *A. viridis* is a non-hyperaccumulator of As. Hence, it possesses lower potential for phytostabilization of total-As in its root and shoot tissues, while the phenomenon is remarkable in Chinese brake fern [2, 13]. When PO_4^{3-} was amended with the AsO_4^{3-} supplies, it resulted in the increase in total chlorophyll as well as biomass. For example, the pigments increased 3.84 times, along with 3.86 and 1.57 folds rise in biomass of the root and shoot tissues, respectively at 40 µM AsO_4^{3-} amended with 400 µM PO_4^{3-}. The moderating effect of PO_4^{3-} amendments on AsO_4^{3-} toxicities, with respect to total chlorophyll and biomass was further confirmed by significant ($p < 0.05$) direct correlation (Fig. 3c, d). Similar effect was observed by Tu and Ma [2], where linear and quadratic model was proposed for the interaction of P, along with pH and AsO_4^{3-} supply on Chinese brake fern (*Pteris vittata* L.).

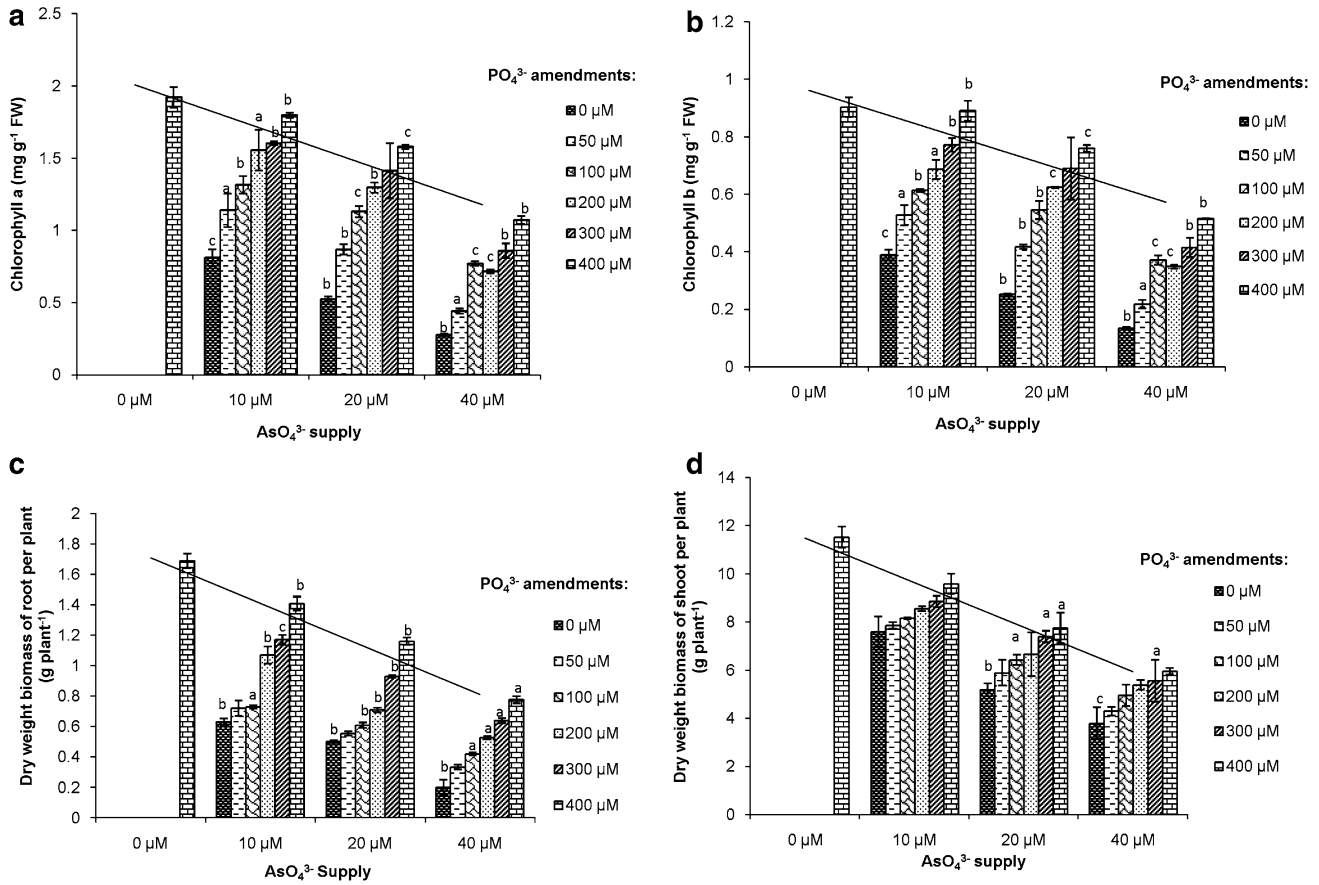

Fig. 2 Variations of **a** chlorophyll a and **b** chlorophyll b in leaves; **c** biomass (g plant^{-1} DW) of root and **d** shoot tissues of *A. viridis* with various AsO_4^{3-}/PO_4^{3-} amendments at 28 days of experiment. *Error bars* represent SE of means (n = 3). The alphabets *a*, *b* and *c* indicate values that differ significantly from control at $p < 0.05$; $p < 0.01$ and $p < 0.001$, respectively. Columns without any of the alphabets represent differently significant results

Total P concentration was greater (nearly sixfold) in shoot than in root tissues of *A. viridis* (Fig. 4a, b). This difference in P accumulation might be due to the fact that various metabolic activities like photosynthesis, electron transport etc. occur in the aerial parts of the plant, resulting in the translocation of P to these parts [34]. This, in turn, was responsible for higher accumulation of P and lower accumulation of total-As in shoot tissues than in root of *A. viridis*. In roots (Fig. 4a), AsO_4^{3-} supplies reduced the P accumulation to the maximum of 94.31 %, observed at 40 µM AsO_4^{3-} supply, deficient in PO_4^{3-}. Similarly, in the shoot tissues (Fig. 4b), the highest reduction was 95.65 % under the above conditions. Significant ($p < 0.05$) negative correlation ($r = -0.85$ for root; $r = -0.76$ for shoot) between P accumulation and total-As accumulation strongly supported this interaction (Fig. 1c). Furthermore, the extent of P-deficiency in the root and shoot tissues of *A. viridis* may also be correlated with the severity of decrease in the respective biomass. Such observation was reported in *Pistia stratiotes* L. under hydroponic conditions [12]. The antagonistic interactions between AsO_4^{3-} and PO_4^{3-} uptake were similar to the other works [3, 10, 13, 14, 28]. The total P accumulations in root and shoot tissues increased in dose-dependent manner, irrespective of AsO_4^{3-} concentration, with the maximum enhancements of 5.29 and 3.79 folds, respectively.

Total N accumulation in root tissues exceeded the shoot tissues of *A. viridis,* with/without AsO_4^{3-}–PO_4^{3-} amendments. This observation may be reasoned as, the nitrate is taken up by the roots and majority of it gets reduced, assimilated and stored in vacuoles. The remaining translocates into shoot parts for the further metabolism, as confirmed in the corn seedlings [35]. With the introduction of AsO_4^{3-} into the growth medium, the amount of total N stored in root (Fig. 5a) and shoot (Fig. 5b) tissues increased in dose-dependent manner. In PO_4^{3-} deficient system the maximum increase in total N contents in root tissue was 6.32-fold, while 3.05-fold increase was observed for shoot tissue at 40 µM AsO_4^{3-} supply. The increments of total N accumulation in root and shoot tissues with

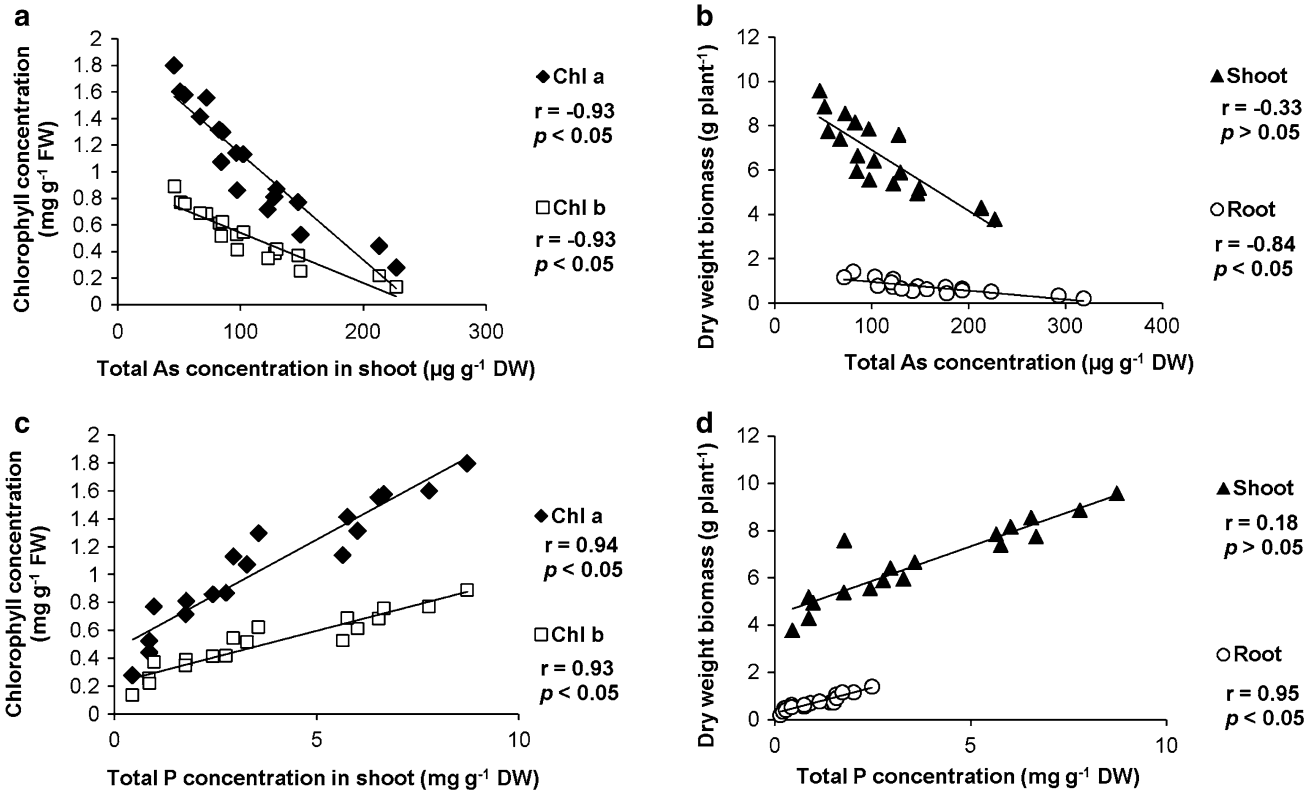

Fig. 3 Relationships between **a** chlorophyll concentration and As accumulation in shoot tissues, **b** dry weight biomass and total As accumulation in root and shoot tissues, **c** chlorophyll concentration and total P accumulation in shoot tissues, and **d** dry weight biomass and total P accumulation in root and shoot tissues of *A. viridis,* each with representations of Pearson's correlation coefficient (r-value) and level of significance (*p* value)

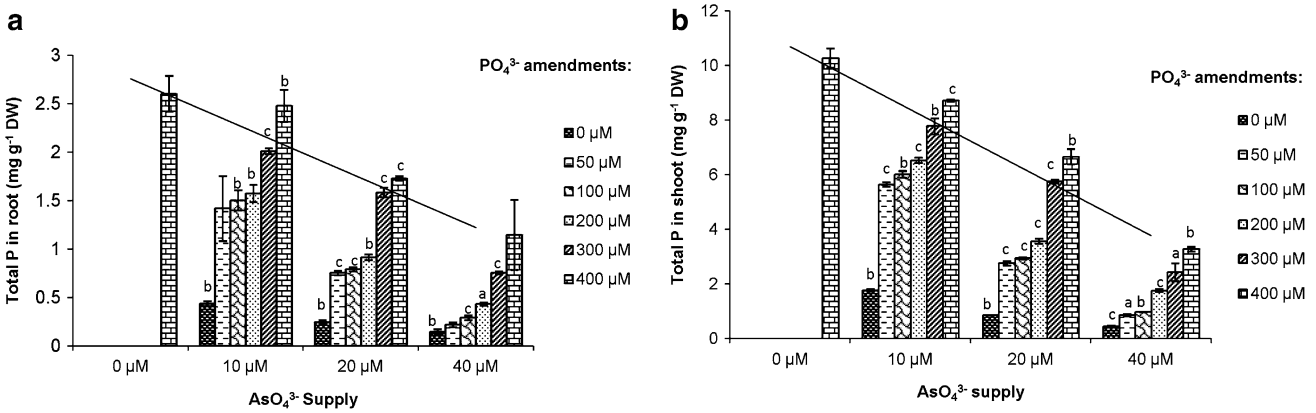

Fig. 4 Variations of total P accumulations (mg g^{-1} DW) in **a** root and **b** shoot tissues of *A. viridis* with various AsO_4^{3-}/PO_4^{3-} amendments at 28 days of experiment. *Error bars* represent SE of means (n = 3). The alphabets *a*, *b* and *c* indicate values that differ significantly from control at $p < 0.05$; $p < 0.01$ and $p < 0.001$, respectively. Columns without any of the alphabets represent differently significant results

respect to AsO_4^{3-} supplies were statistically supported by their direct significant (at 0.05 level) relationships (Fig. 5c). Generally, N is an essential nutrient for growth and metabolism (such as photosynthesis) and also an inseparable component of enzymes. Hence, it will be of no surprise to consider that metal toxicity will reduce the N accumulation [36]. However, the present study reports the opposite effect. Such observation may be reasoned as the

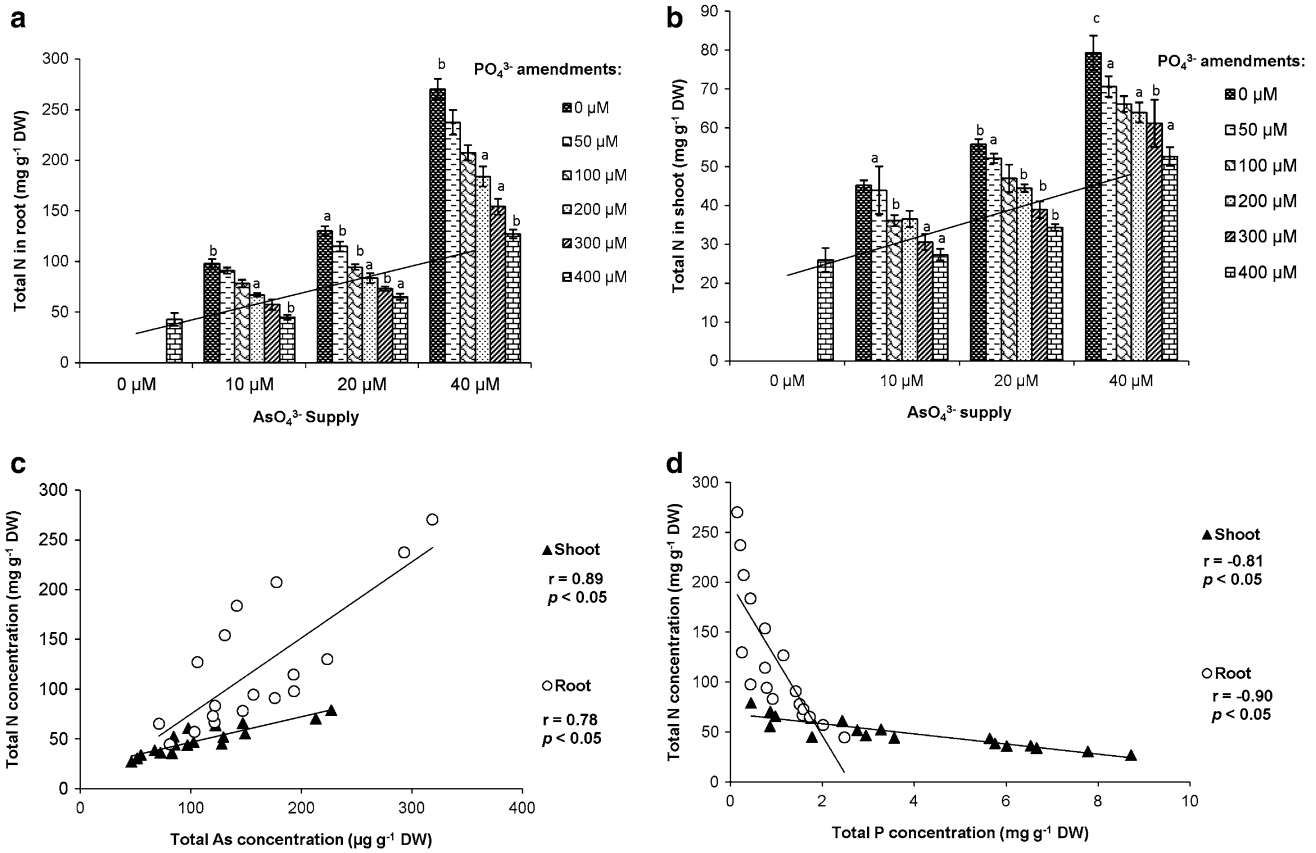

Fig. 5 **a** Variations of total N accumulations (mg g^{-1} DW) in root and **b** shoot tissues of *A. viridis* with various AsO_4^{3-}/PO_4^{3-} amendments at 28 days of experiment. *Error bars* represent SE of means (n = 3). The alphabets *a*, *b* and *c* indicate values that differ significantly from control at $p < 0.05$; $p < 0.01$ and $p < 0.001$, respectively. Columns without any of the alphabets represent differently significant results; **c** relationships between total N and total As accumulations, and **d** total N and total P accumulations in root and shoot tissues of *A. viridis*, each with representations of Pearson's correlation coefficient (r-value) and level of significance (*p* value)

increase in metal concentration would have caused increased enzymatic activities (esp. antioxidant enzymes) to combat the metal induced oxidative stress, as also reported in *Ipomoea reptans* [6]. Furthermore, the reduction in the toxicity of AsO_4^{3-} due to PO_4^{3-} amendments is observed as reduction in N accumulation. The PO_4^{3-} amendments discouraged the accumulation of total N levels in both root (Fig. 5a) and shoot (Fig. 5b) tissues with the maximum of 39.55 and 54.23 %, respectively at 10 µM AsO_4^{3-} supply, amended with highest concentration (400 µM) of PO_4^{3-}. Reciprocal correlation (Fig. 5d) between the increased PO_4^{3-} accumulation and total N accumulation in root and shoot tissues further added to the knowledge regarding the benefits of PO_4^{3-}.

Any heavy metal stress results in the production of reactive oxygen species (ROS) in plants. The plants respond to this stress by producing necessary antioxidant enzymes like SOD, CAT and POD to reduce the ROS into harmless products like water and molecular oxygen. Hence, the present investigation involved the estimation of the above key antioxidant enzymes in the root and shoot tissues of *A. viridis,* that were involved in combating the oxidative stress, generated due to AsO_4^{3-} treatments. As an obvious fact, the increase in AsO_4^{3-} concentration resulted in the notable increment in the levels of SOD, CAT and POD. Since, the root accumulated higher amount of total-As than shoot, the general trend of antioxidant enzyme levels also indicated the same differences. In addition, the level of enzymes increased with incubation time. In the root as well as shoot tissues, the maximum activities of SOD (Fig. 6a, b), CAT (Fig. 6c, d) and POD (Fig. 7a, b) were observed at 40 µM AsO_4^{3-}–0 µM PO_4^{3-} plants at the end of 12 h incubation with respect to control. The correlation of antioxidant enzymes in root and shoot tissues, with respect to increased dosage of AsO_4^{3-} (Supplementary Information Tables S1a and b; $0.90 \leq r \leq 0.99$ for root tissues and $0.72 \leq r \leq 0.99$ for shoot tissues, respectively) and time intervals (Supplementary Information Tables S2a and b; $0.70 \leq r \leq 0.99$ for root tissues and $0.69 \leq r \leq 0.99$ for shoot tissues, respectively) were

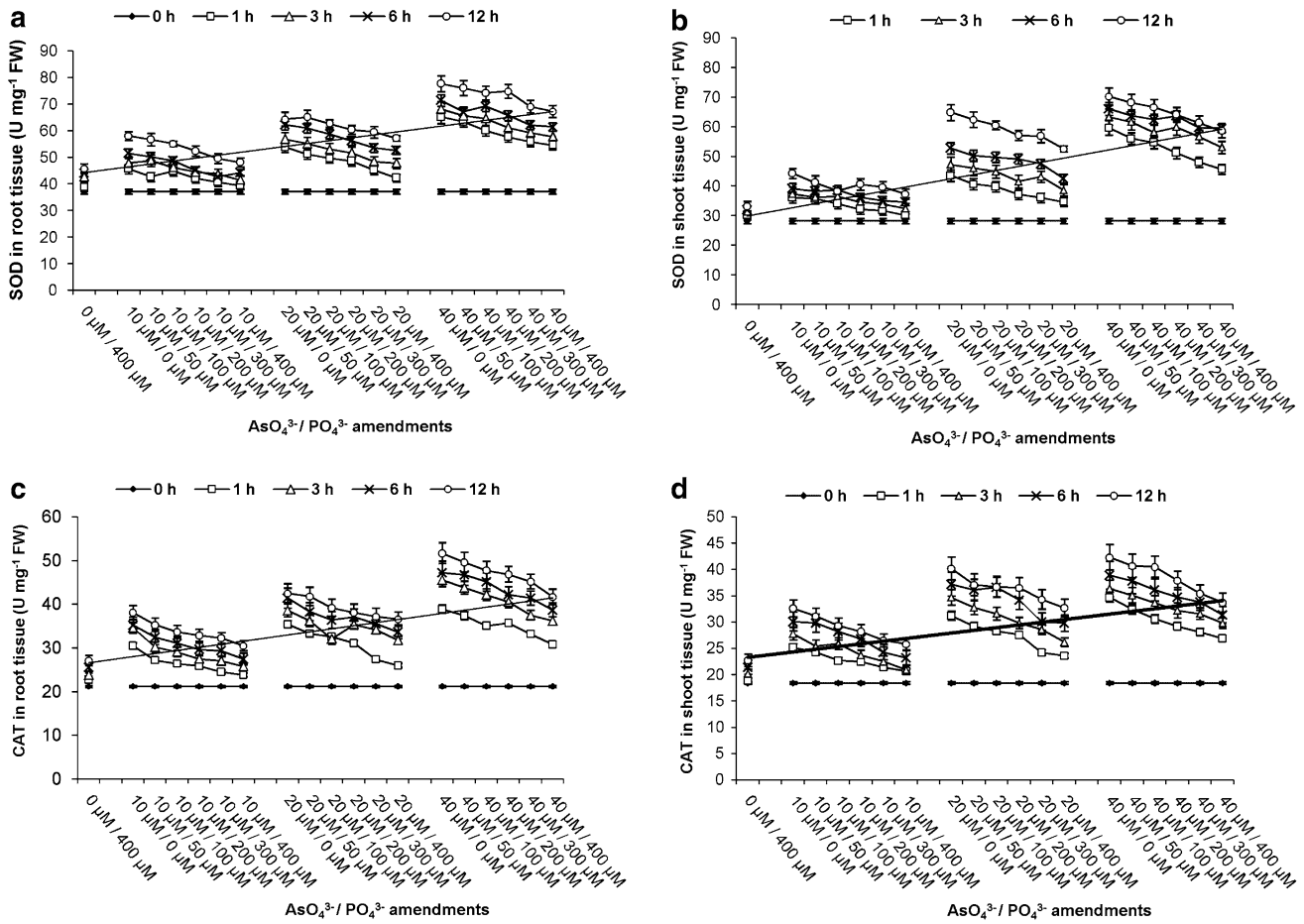

Fig. 6 Variations of **a** superoxide dismutase (SOD) activities (U mg^{-1} FW) in root and **b** shoot tissues; **c** catalase (CAT) activities (U mg^{-1} FW) in root and **d** shoot tissues of *A. viridis* for time intervals 0, 1, 3, 6 and 12 h with various AsO_4^{3-}/PO_4^{3-} amendments at the first day of experiment. *Error bars* represent SE of means (n = 3)

examined, which resulted into highly significant positive r-values. In the shoot tissues, the increase of SOD and POD was 2.12 and 1.47 folds, respectively. The study further indicated that the degree of SOD-increment was higher than that of CAT (1.86-fold) and POD levels. The dominance of SOD over the other two antioxidant enzymes may be reasoned that SOD itself functions as the key enzyme to act upon more toxic O_2^- ion, whereas CAT and POD, together act upon the 'SOD-generated' less toxic H_2O_2 from the previous reaction [1, 4, 6]. The present study was comparable to the work on red clover plants (*Trifolium pratense* L.), in which, the plants showed an increase in SOD and POD activities in shoot tissues, up to twofolds each when the concentration of the As supply was increased from 5 to 10 mg kg^{-1} [1]. Similarly, in Chinese brake fern, chickpea and Indian mustard the increasing supply of As resulted in the increased SOD and CAT activities [3, 24, 37]. PO_4^{3-} amendments caused a significant lowering of antioxidant enzyme activities in both root and shoot tissues of *A. viridis,* in the dose-dependent manner. The maximum decrease in SOD in root (Fig. 6a) was 21.52 %, while, in case of CAT, the maximum decrease was 26.55 % (Fig. 6c). The range of reduction of POD levels with respect to the respective controls in root (Fig. 7a) was 6.33–28.44 % and in shoot (Fig. 7b) it was 13.45–22.07 %. The reciprocal correlation between the PO_4^{3-} amendments and antioxidant enzymes has been shown in the Supplementary Information Tables S3a and b ($-0.99 \leq r \leq -0.68$ for root tissues and $-0.99 \leq r \leq -0.74$ for shoot tissues, respectively) depicting negative r-values. The present study is consistent with the reduced levels of CAT (42.86 %) and POD (26.42 %) in chickpea [3]. Due to the reduced accumulation of total-As during PO_4^{3-} amendments, the reduction in the antioxidant enzymes in root and shoot tissues of *A. viridis* was obvious [2, 14]. Hence, the reduced level of antioxidant enzymes served as the confirmation of the hypothesis that PO_4^{3-} amendments moderate AsO_4^{3-} toxicity in *A. viridis.*

Similar to antioxidant enzyme level, the degree of lipid peroxidation (in terms of malondialdehyde or MDA

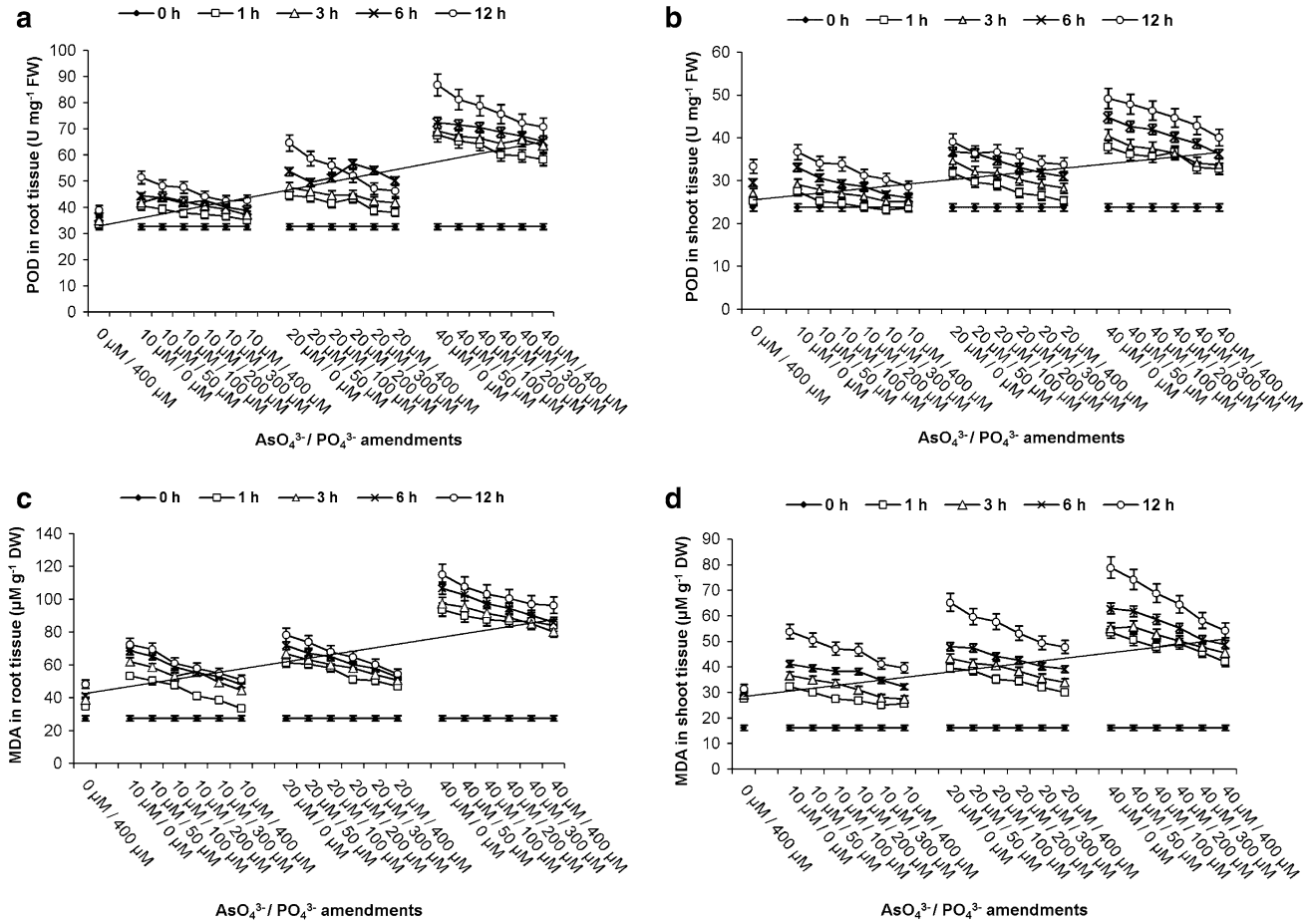

Fig. 7 Variations of **a** peroxidase (POD) activities (U mg^{-1} FW) in root and **b** shoot tissues; **c** lipid peroxidation (malondialdehyde or MDA content expressed as μM g^{-1} DW) levels in root and **d** shoot tissues of *A. viridis* for time intervals 0, 1, 3, 6 and 12 h with various AsO_4^{3-}/PO_4^{3-} amendments at the first day of experiment. *Error bars* represent SE of means (n = 3)

content) in the plant tissues is also an indicator of heavy metal dependent oxidative toxicity [6]. The mechanism mainly involves the ROS mediated removal of a hydrogen atom from the carbon of unsaturated fatty acid molecules of the lipid bilayer. This allows the oxygen to bind in the vacant position, resulting in the formation of reactive lipid peroxy radical, and further the same kind of reaction is propagated [4, 6]. During the course of reactions, MDA is generated, which is when determined, to assess the degree of lipid peroxidation. As in the present study, due to the higher accumulation of total-As, root tissues (Fig. 7c) showed an average of 1.5-fold higher MDA content than shoot (Fig. 7d), irrespective of AsO_4^{3-} supplies and incubation periods. The maximum increase in MDA level in root and shoot tissues were 3.66 and 2.5 folds, respectively, at 40 μM AsO_4^{3-} supplied without PO_4^{3-} amendment and 12 h incubation period. As per the correlation statistics, levels of MDA in root and shoot tissues were directly related to AsO_4^{3-} (Supplementary Information Tables S1a and b; $0.96 \leq r \leq 0.99$ for root tissues and $0.89 \leq r \leq 0.99$ for shoot tissues, respectively) supplies and time of incubation (Supplementary Information Tables S2a and b; $0.64 \leq r \leq 0.98$ for root tissues and $0.58 \leq r \leq 0.94$ for shoot tissues, respectively). In comparison to the MDA levels of chickpea [3], the present study reports twice the lipid peroxidation, indicating the sensitivity of leafy vegetable towards abiotic stress. Other crop-plants, like *Phaseolus vulgaris*, *Helianthus annus*, *Pisum sativum* etc., also showed an increased lipid peroxidation in the presence of heavy metal stress [38–40]. Lipid peroxidation decreased in the PO_4^{3-} amended *A. viridis* with respect to the PO_4^{3-} deficient samples at each AsO_4^{3-} treatment. In the root tissues (Fig. 7c), a drastic decrease in the lipid peroxidative damage was observed in the first hour of incubation, since, MDA concentration decreased up to 37.15 % at 10 μM AsO_4^{3-} supply amended with 400 μM. However, in shoot tissues (Fig. 7d), the maximum reduction (31.18 %) of MDA level was observed at 40 μM

AsO_4^{3-} treated with 400 μM PO_4^{3-} and 12 h incubation period. The decrease in the MDA level in root and shoot tissues with respect to increasing PO_4^{3-} amendments was statistically justified by correlation analysis (Supplementary Information Tables S3a and b; $-0.99 \leq r \leq -0.89$ for both root and shoot tissues, respectively).

Conclusion

Owing to the toxicity of arsenate in the leafy vegetable, *Amaranthus viridis* at 40 μM of AsO_4^{3-} stress, there was a mean enhancement up to 182 % of antioxidant enzymes and MDA levels. However, under the combination of AsO_4^{3-}–PO_4^{3-} supplies, the average decrease in the antioxidant enzymes and MDA was 28.42 %. The results also indicate that more than 25 % of AsO_4^{3-} can be prevented to enter into the crops by the PO_4^{3-} amendment in their growth medium leading to reduction in the toxicities at physiological level. In conclusion to these findings, it may be suggested that this strategy may help to moderate the entry of arsenic into the food chain via crop plants.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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