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RESEARCH ARTICLE

Arsenate and arsenite-induced inhibition and recovery in two diazotrophic cyanobacteria *Nostoc muscorum* and *Anabaena* sp.: study on time-dependent toxicity regulation

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Abstract

Exposure time, metal bio-accumulation, and upregulation of ascorbate-glutathione (AsA-GSH) cycle are the key factor that provide tolerance against heavy metal stress. Thus, the current study is an endeavor to prove our hypothesis that regulation of arsenate (As^V: 50, 100, and 150 mM) and arsenite (As^{III}: 50, 100, and 150 μM) toxicity is time dependent (48–96 h) due to modulation in bio-accumulation pattern, AsA-GSH cycle, and non-enzymatic antioxidants in two paddy field cyanobacteria *Nostoc muscorum* ATCC27893 and *Anabaena* sp. PCC7120. After 48 h, reduction in growth associated with increased sensitivity index, As bio-accumulation, and oxidative stress was observed which further intensified after 96 h but the degree of damage was lesser than 48 h. It denotes a significant recovery in growth after 96 h which is correlated with decreased As bio-accumulation and oxidative stress due to increased efficiency of AsA-GSH cycle and non-enzymatic antioxidants. Both the species of As caused significant rise in oxidative biomarkers as evident by in -vitro analysis of $O_2^{\cdot-}$, H_2O_2, and MDA equivalent contents despite appreciable rise in the activity antioxidative enzymes APX, DHAR, and GR. The study concludes that among both forms of arsenic, As^{III} induced more toxic effect on growth by over-accumulating the ROS as evident by weak induction of AsA-GSH cycle to overcome the stress as compared to As^V. Further, with increasing the time exposure, apparent recovery was noticed with the lower doses of As^V, i.e., 50 and 100 mM and As^{III}, i.e., 50 and 100 μM; however, the toxicity further aggravated with higher dose of both As^V and As^{III}. Study proposes the deleterious impact of As^V and As^{III} on cyanobacteria *N. muscorum* and *Anabaena* sp. but the toxicity was overcome by time-dependent recovery.

Keywords Arsenic accumulation · Ascorbate-glutathione cycle · Growth · Oxidative biomarker · Isoenzyme patterning

Introduction

Contamination of ecosystems with toxic metals is a global environmental issue and arsenic (As) contamination is one of them (Zheng and Ayotte 2015). Arsenic (As) is a ubiquitous toxic metalloid, percolates in the environment either via natural events such as geochemical weathering of rocks, volcanic eruptions, microbial action, or man-made actions like mining, smelting of ore, use of As-based fertilizers, pesticides (arsenicals), semi-conductors, lead-acid batteries, and fly ash disposals (Chen and Rosen 2016). Arsenic exist in both inorganic and organic forms with oxidation state ranging from − 3 to + 5 and inorganic forms are more toxic (Jedynak et al. 2010; Miyashita et al. 2015). Among them, most stable inorganic forms of As are arsenate (As^V) and arsenite (As^{III}) that adversely affect the growth and development of various life forms, including microorganism such as cyanobacteria (Patel et al. 2018). Being phosphate analogue, As^V uses phosphate transporters for their uptake and impairs the phosphorylation processes (ATP synthesis) (Rensing and Rosen 2009) and electron transport chain. However, As^{III} uses aquaglyceroporins (AQGP) for their uptake and damages the macromolecule and membrane proteins having sulfhydryl

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groups (–SH) and thus forces greater toxicity than As^V (Rensing and Rosen 2009). Similar to other heavy metals, both forms of As are well known to generate reactive oxygen species (ROS) that causes oxidative stress which alters various physiological and biochemical processes (Patel et al. 2018).

Oxidative stress leads to degradation of membrane which eventually leads to cell damage and ultimately cell death (Gebel 2000), and also disrupts the production of metabolite such as ascorbate (AsA) and glutathione (GSH). Disparity in the ratio of AsA/GSH eventually decreases the efficiency of AsA-GSH cycle (important role in H_2O_2 detoxification) (Bhattacharjee et al. 2008). Being high stability and permeability across the membrane, H_2O_2 is not effectively detoxified by peroxidases; hence, AsA-GSH cycle plays a defensive role under oxidative stress (Kumar et al. 2017). Detoxification of H_2O_2 via AsA-GSH cycle begun with the action of ascorbate peroxidase (APX) that reduces H_2O_2 into H_2O by taking electrons form AsA and replenishment of AsA is done by dehydroascorbate reductase (DHAR) at the expense of GSH and converting it into GSSG (Borella et al. 2019). Further, GSSG is transformed into GSH by accepting electron from NADPH through NADPH-dependent glutathione reductase (GR). The AsA and GSH function as low-molecular weight non-enzymatic antioxidants and also involve in the synthesis of phytochelatins (PCs) (Begum et al. 2016) and direct a signal response for the regulation of antioxidative mechanisms (Chattergee et al. 2018). Apparently other non-enzymatic antioxidants like proline, cysteine, and non-protein thiols also participate in direct quenching of ROS. Decrease in the efficiency of AsA-GSH cycle directly linked to alterations in growth and other physiological processes concomitant with increased oxidative stress.

Cyanobacteria (blue green algae; BGA) are major inhabitants of paddy fields because paddy field provides suitable environment for their growth and in turn cyanobacteria fulfill the nitrogen requirement of soil by fixing atmospheric nitrogen in the form of nitrate (NO_3^-) (Whitton 2000). Besides this, they are good source of protein, secondary metabolites, and other compounds having medicinal or pharmaceutical properties Singh et al. (2016a, b). Major inhabitants of paddy fields are *Anabaena variabilis*, *Nostoc muscorum*, *Aulosira fertilissima*, and *Tolypothrix tenuis* and are well-known biofertilizers as they enhance the crop productivity by 30% by fixing around 20–30 kg N ha^{-1} (Zehr 2011). Cyanobacteria have faced adverse environmental conditions due to their aquatic habitat and have inheriting ability to cope with these stress factors (Perales-Vela et al. 2006). Arsenic enforced deleterious impact on cyanobacteria by affecting growth, pigment content, and nutrient imbalance and leads to the oxidative stress associated with increase antioxidant defense system (Upadhyay et al. 2016; Patel et al. 2018) but some genera such as *Synechocystis*, *Oscillatoria*, *Anabaena*, and *Phormidium* flourished in metal-contaminated sites as they have capability to accumulate toxic metals such as As and function as bioremediants (Kulp et al. 2008; Ferrari et al. 2013). Tolerance and survival of cyanobacteria at such higher As concentration indicates towards its decontamination efficiency and strategy to overcome As stress due to conversion of toxic forms into less toxic forms via volatilization/bio-methylation. Thus, the present work has been undertaken to elucidate differential response of As^V and As^{III} on two cyanobacteria by evaluating the growth, bio-accumulation, oxidative stress, and efficiency of AsA-GSH cycle under two different time intervals (48 h and 96 h) and to illuminate time-dependent recovery.

Materials and methods

Test organisms and culture conditions

The cyanobacteria *Nostoc muscorum* and *Anabaena* sp. PCC7120 were grown in BG-11 medium (pH = 7.5) in a well temperature maintained culture room with a light/dark regime of 14:10 h and at 25 ± 2 °C temperature under 75 μmol photons m^{-2} s^{-1}.

Treatment designing

Sodium arsenate ($Na_2HAsO_4{\cdot}7H_2O$) and sodium arsenite ($NaAsO_2$) were used as a source of arsenate (As^V) and arsenite (As^{III}). Screening experiment was performed (with 0.1 OD) culture for the dose selection; As^V at 25, 50, 100, 150, and 200 mM and As^{III} at 25, 50, 100, 150, and 200 μM were screened and of the above three doses of As^V, 50, 100, and 150 mM and As^{III}, 50, 100, and 150 μM were selected for the detail study (published in Patel et al. 2018). The selected doses of As^V (50, 100, and 150 mM) were found to inhibit the growth of *N. muscorum* by 10, 30, and 50% and by 15, 33, and 56% for *Anabaena* sp. respectively. Under similar conditions, selected doses of As^{III} (50, 100, and 150 μM) were found to inhibit the growth of *N. muscorum* by 14, 35, and 54% and by 20, 40, and 60% for *Anabaena* sp. respectively (Patel et al. 2018). After 48 and 96 h of experiments, different parameters were analyzed.

Measurement of growth

Growth was measured in the terms of RGR and sensitivity index.

$$\text{Relative growth rate (RGR) } d^{-1} = \frac{\text{In } W_2\text{-In } W_1}{T_2\text{-}T_1}$$

where

$\ln W_2$ natural logarithm of dry weight [μg (mL culture)$^{-1}$] at time T_2

$\ln W_1$ natural logarithm of dry weight at time T_1

T_1 initial time
T_2 final time

The sensitivity index (SI) of the test seedlings was calculated with the help of the following equation:

$$SI_{treatment}\ (\%) = 100 \times \left[DM_{treatment} - DM_{control})/DM_{control}\right]$$

(DM = dry mass)

Intracellular accumulation of As

The cellular accumulation of As was estimated by atomic absorption spectrophotometer (iCE 3000 series, Model 3500 AAS, Thermo scientific, UK); the instrument was calibrated with the graded solution of As. The samples were prepared by harvesting and centrifuging 80 ml treated culture, and washed with 1 mM EDTA and suspended in chilled phosphate buffer and centrifuged and the pellets were oven dried at 80 °C for 3 days. Dried cells were digested after adding tri-acid mixture (HNO_3, H_2SO_4, and $HClO_4$) in ratio of 5:1:1 at 80 °C until a transparent solution obtained.

Biochemical analysis of oxidative stress biomarkers

Oxidative stress biomarkers: superoxide radicals (SOR; $O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) were estimated by following the method of Elstner and Heupel (1976) and Velikova et al. (2000), respectively. Moreover, indices of oxidative stress as lipid peroxidation (measured in terms of malondialdehyde; MDA equivalent contents) were estimated by the method of Heath and Packer (1968) (sample preparation mentioned and published in Patel et al. 2018).

Ascorbate-glutathione cycle

Ascorbate-glutathione cycle enzymes: ascorbate peroxidase (APX), glutathione reductase (GR), and dehydroascorbate reductase (DHAR) activity

The method of Nakano and Asada (1981) was followed for estimation of APX activity in treated and untreated cyanobacterial suspension. The pellets were homogenized in phosphate buffer (pH 7.0) containing EDTA and reduced ascorbate, followed by centrifugation and were mixed with reaction mixture containing EDTA, reduced ascorbate, and H_2O_2. The absorbance was recorded at 290 nm by using UV-Visible Double beam-1700 Spectrophotometer, Shimadzu, Japan. One unit of enzyme activity is defined as 1 nmol ascorbate oxidized min^{-1}.

The glutathione reductase activity was assayed by following the method of Schaedle and Bassham (1977). By homogenizing the pellets in phosphate buffer containing EDTA, and adding reaction mixture prepared in phosphate buffer containing EDTA, NADPH, GSSG, and enzyme extract, the absorbance due to oxidation of NADPH was recorded at 340 nm. One unit of enzyme activity is defined as 1 nmol NADPH oxidized min^{-1}.

The DHAR activity was measured by following the method of Nakano and Asada (1981). Sample extract was mixed with reaction mixture containing potassium phosphate buffer (pH 7.0) EDTA, GSH, and DHA. The absorbance due to the reduction of DHA into AsA was recorded at 265 nm using UV-Visible Double beam-1700 Spectrophotometer, Shimadzu, Japan. One unit of enzyme activity is defined as 1 nmol DHA reduced min^{-1}.

Isoenzyme profiling of ascorbate peroxidase (APX) and glutathione reductase (GR)

The native-PAGE profiling of APX and GR was performed on discontinuous polyacrylamide gels (PAGE) with 4.5% polyacrylamide in stacking with 10% separating gel. Initially proteins were electrophoretically separated at 4 °C. After running the electrophoresis, detection of isoenzyme was performed by staining method. For APX, the 10% gel was pre-run in tank buffer containing 2 mM ascorbate for 30 min. After pre-running, samples were loaded in gels and running completed in normal tank buffer. After running, the gel was stained by adopting the method of Mittler and Zilinskas (1993). Similarly in GR, the gel was stained by following the methods of Ye et al. (1997). The photographs were captured with Nikon, Coolpix S3100, Japan.

Metabolites of ascorbate–glutathione cycle

Estimation of total ascorbate (AsA+DHA), reduced ascorbate (AsA), and oxidized ascorbate (dehydroascorbate: DHA)

By following the method of Gossett et al. (1994), total ascorbate (AsA+DHA), reduced ascorbate (AsA), and dehydroascorbate (DHA) contents were determined. The homogenate was centrifuged at 22,000*g* for 15 min. The total ascorbate content was determined in a reaction mixture consisting of supernatant, potassium phosphate buffer (pH 7.4) containing EDTA, and DTT that reduces DHA to AsA. The AsA was assayed similarly except that DTT was replaced by 0.2 ml of deionized H_2O. The DHA was determined by subtracting AsA from total ascorbate. Ascorbate content was quantified by using standard curve prepared with L-ascorbic acid.

Estimation of reduced glutathione (GSH) and oxidized glutathione (GSSG) and total glutathione (GSH+GSSG)

Total, reduced, and oxidized glutathione were determined by adopting the method of Brehe and Burch (1976) with some modifications. The absorbance was recorded at 412 nm for 5

min. The GSSG was assessed by incubating enzyme extract with 2-vinylpyridine for 1 h at 25 °C followed with vigorous shaking. The amount of GSH was determined by subtracting GSSG from total glutathione using a standard curve prepared with GSH.

Estimation of non-enzymatic antioxidant

Estimation of proline (Pro) and cysteine (Cys) content

Estimation of proline and cysteine content was done by the method of Bates et al. (1973) and Gaitonde (1967), respectively. For Pro content, the cells were harvested and the pellets obtained were crushed in 3% sulphosalicylic acid, and for Cys samples homogenized in 5% chilled perchloric acid were centrifuged and the supernatant is added with reaction mixture. The samples were kept for water bath at 95 °C for 1 h, then cooled and extracted with 4 ml toluene. The absorbance was read at 520 and 560 nm respectively. The Pro and Cys content in each sample was calculated from the standard curve.

Estimation of non-protein thiol (NP-SH) and total phenolic (TPCs)

For NP-SH estimation, cultures were centrifuged and the pellets were homogenized in sulphosalicylic acid and content was estimated by taking absorbance at 412 nm by following the method of Ellmann (1959).

Total phenolic content (TPC) was estimated following the method of Waterhouse (2001). The culture was homogenized in ethanol and centrifuged at 4 °C. The absorbance was recorded at 765 nm compared with a standard curve prepared by gallic acid. Unit is expressed as [μg gallic acid equivalents (GAE) (mg^{-1} dry weight)].

Statistical analysis

The results were statistically analyzed by analysis of variance (ANOVA). Duncan multiple range tests (DMRT) was applied for mean separation to show significant differences among treatments at $p < 0.05$ significance level (SPSS 16.0). The results presented are means ± standard error of three replicates ($n = 3$). Pearson correlation coefficients were calculated to show the differential action of As^V and As^{III} on different physiological and biochemical parameters using correlation.

Experimental findings

Growth

Growth behavior of selected cyanobacteria, i.e., *Nostoc muscorum* and *Anabaena* sp. treated with varying doses of arsenate (As^V; 50, 100, and 150 mM) and arsenite (As^{III}; 50, 100, and 150 μM) was monitored by observing changes in growth-related parameters, i.e., relative growth rate and sensitivity index. The results pertaining to these parameters have been portrayed in Fig. 1 a and b. The growth significantly declined by 9%, 29, and 47% under As^V exposure and by 13%, 34, and 52% under As^{III} after 48 h of exposure further after 96 h the extent of toxicity was reduced with lower doses as recovery in growth was observed. Similar declining trend was observed for *Anabaena* sp. but the damage was more prominent compared to *N. muscorum*. Sensitivity index also corroborated the growth result and showed increased sensitive nature with increasing As concentration and more sensitivity towards As^{III} treated cultures of both the species while greater sensitivity was recorded in *Anabaena* sp.

Arsenic accumulation

Accumulation of arsenic was found to increase parallelly with increasing As in medium. The intracellular accumulation of As in As^{III} treated cells was higher than As^V species as shown in Table 1. After 48 h, *N. muscorum* at 50, 100, and 150 mM of As^V accumulated 0.142 ± 2.4, 0.334 ± 5.7, and 0.482 ± 8.3 mg As g^{-1} dry weight while *Anabaena* cells accumulated 0.163 ± 3.4, 0.401 ± 4.7, and 0.587 ± 6.3 mg As g^{-1} dry weight, further on As^{III} exposure the value for intracellular arsenic accumulation was increased. Contrastingly, after 96 h, the intracellular accumulation of As was found to decline at lower doses of As^V (50 and 100 mM) and As^{III} (50 and 100 μM). While further enhancement at higher doses, i.e., 150 mM of As^V and 150 μM As^{III} was recorded, in comparison to the data recorded at 48 h of treatment. Moreover, the intracellular As accumulation was higher under As^{III} treatment at all the test doses in comparison to As^V treatment. The arsenic content was not detected in control samples.

Oxidative stress biomarkers: (SOR, H_2O_2, and lipid peroxidation)

The results pertaining to in -vitro $O_2^{\cdot-}$ and H_2O_2 content in test cyanobacteria exposed to As^V and As^{III} have been depicted in Fig. 2 a and b. In *N. muscorum*, the $O_2^{\cdot-}$ content was raised by 27, 58, and 72% at 50, 100, and 150 mM of As^V and by 36, 74, and 89% at 50, 100, and 150 μM of As^{III}, respectively, over the control values. Similarly, the H_2O_2 content increased by 35, 68, and 80% at 50, 100, and 150 mM of As^V and 42, 76, and 96% at 50, 100, and 150 μM of As^{III}, after 48 h of exposure. Similar trend was noticed in *Anabaena* sp. under similar condition but the $O_2^{\cdot-}$ and H_2O_2 content was found to be greater than that of *N. muscorum*. Moreover, after 96 h of treatment $O_2^{\cdot-}$ and H_2O_2 content was further raised in As^V and As^{III} treatment in both the test cyanobacteria but the damage was more prominent in As^{III} treated *Anabaena* sp. cells.

The ROS-mediated lipid peroxidation was measured in terms of malondialdehyde (MDA) equivalent content exposed to

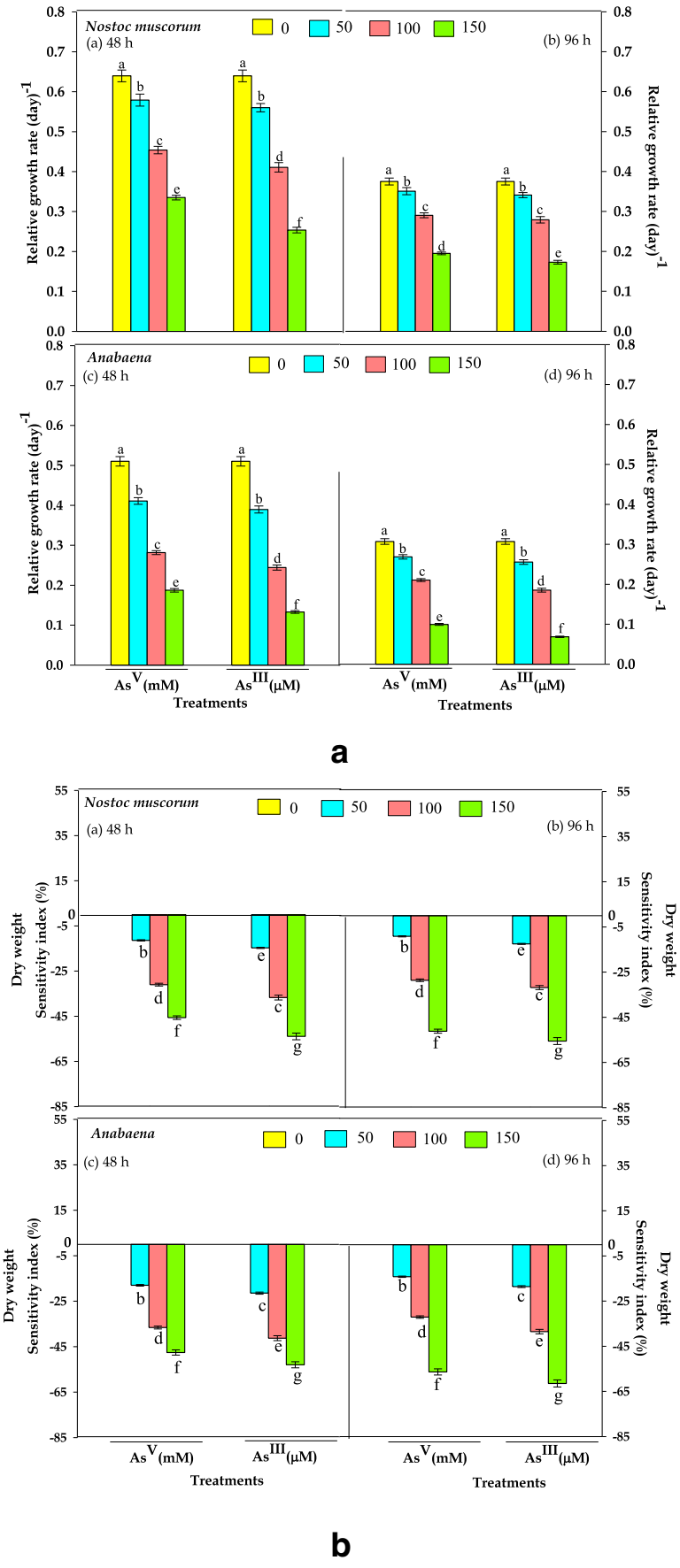


Fig. 1 Effect of arsenate (As^{V}) and arsenite (As^{III}) on **a** relative growth rate and **b** sensitivity index of *Nostoc muscorum* ATCC 27893 and *Anabaena* PCC 7120 after 48 h and 96 h of treatments. Data are means ± standard error of the three replicates ($n = 3$). Bars followed by different letters show significant difference at $P < 0.05$, according to the Duncan multiple range test (DMRT)

Table 1 Intracellular arsenic accumulation in *N. muscorum* and *Anabaena* PCC7120 treated with arsenate (As^V) and arsenite (As^{III}) after 48 and 96 h of experiments

Intracellular As accumulation (mg g^{-1} dry weight)					
Treatment		*Nostoc muscorum*		*Anabaena* PCC7120	
		48 h	96 h	48 h	96 h
Control	0	nd	nd	nd	nd
Arsenate As^V (mM)	50	0.142 ± 0.02^f	0.110 ± 0.02^f	0.163 ± 0.02^f	0.130 ± 0.02^f
	100	0.334 ± 0.05^d	0.280 ± 0.05^d	0.401 ± 0.05^d	0.306 ± 0.05^d
	150	0.482 ± 0.08^b	0.690 ± 0.08^b	0.510 ± 0.08^b	0.783 ± 0.08^b
Arsenite As^{III} (mM)	50	0.191 ± 0.03^e	0.135 ± 0.03^e	0.215 ± 0.03^e	0.152 ± 0.03^e
	100	0.397 ± 0.06^c	0.470 ± 0.06^c	0.440 ± 0.06^c	0.569 ± 0.06^c
	150	0.638 ± 0.10^a	0.876 ± 0.10^a	0.642 ± 0.10^a	0.1190 ± 0.10^a

Data are means ± standard error of three replicates ($n = 3$). Bars followed by different letters show significant difference at $P < 0.05$ significance level according to Duncan multiple range test (DMRT) where *nd* defines as not detected

varying concentration of As^V and As^{III} after 48 and 96 h of experiment (Fig. 2c). The MDA equivalents content was found to be enhanced by 47, 72, and 91% at 50, 100, and 150 mM of As^V and by 56, 86, and 109% at 50, 100, and 150 μM of As^{III} treatment in *N. muscorum* after 48 h. Similar increasing trend for MDA equivalents was observed under As^V and As^{III} treatment in *Anabaena* sp.; however, the damaging effect was greater under As^{III} treated cultures in *Anabaena* sp. as compared to *N. muscorum* after 48 as well as 96 h of treatment.

Furthermore after 96 significant decrease in the degree of oxidative stress biomarkers were noticed with lower doses of both the stress pointing towards the time-dependent recovery against stress condition.

Impact on ascorbate-glutathione (AsA-GSH) cycle

The ascorbate-glutathione cycle is involved in detoxification of H_2O_2. The AsA-GSH cycle involves enzymes such as ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) and its metabolites, i.e., ascorbate (AsA) and glutathione (GSH) present in both oxidized and reduced forms.

(A) Enzymatic activity

Ascorbate peroxidase (APX) activity and its isoenzyme patterning

Results pertaining to APX activity in both the test cyanobacteria have been presented in Fig. 3a. The As^V at 50, 100, and 150 mM doses significantly raised the APX activity by 36, 73, and 61%, in *N. muscorum* and by 28, 63, and 50 % in *Anabaena* sp., respectively, over the control values. Similarly As^{III} at 50, 100, and 150 μM raised the activity by 30, 62, and 54%, in *N. muscorum* and by 20, 51, and 41 % in *Anabaena* sp. after 48 h of the exposure. Moreover, the APX activity was further raised after 96 h of treatment at lower doses of As^V (50 and 100 mM) and As^{III} (50 and 100 μM), while lesser increase at higher doses of As^V (150 mM) and As^{III} (150 μM) was observed but still greater than control.

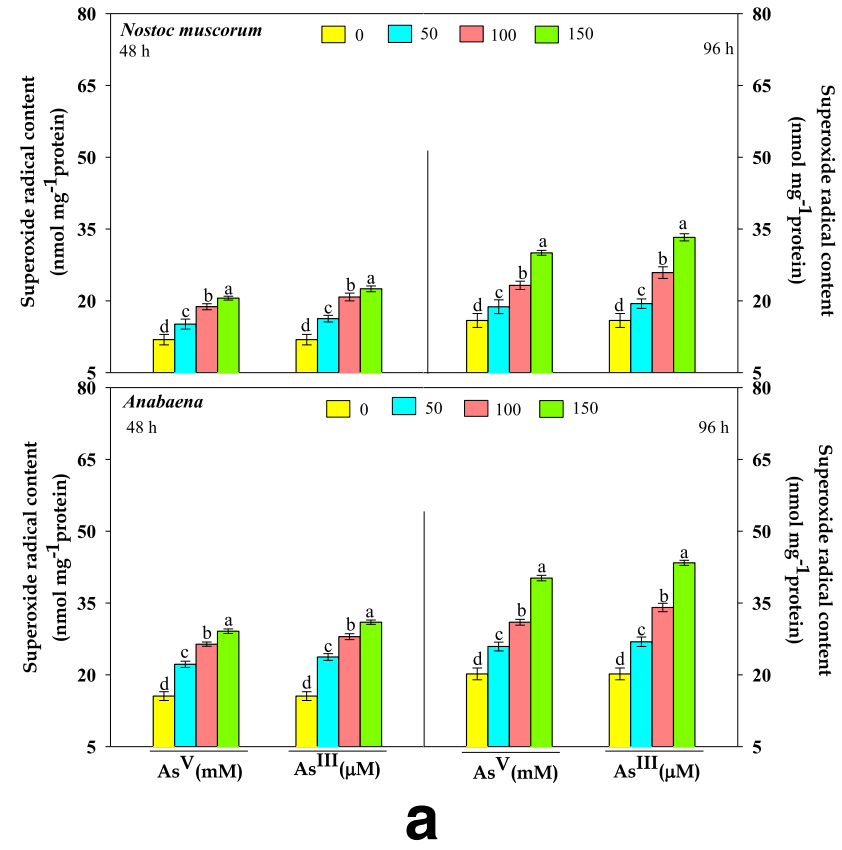
Result pertaining to APX isoenzyme showed two isoforms *viz*: APX I and II in *N. muscorum* while *Anabaena* sp. showed only one isoform, i.e., APX I (Fig. 3d). The results corroborate with the biochemical test.

Dehydroascorbate reductase (DHAR) activity

The DHAR activity in test cyanobacteria treated with As^V and As^{III} has been depicted in Fig. 3b. The results related to DHAR activity reveal that the activity was enhanced by 47, 74, and 66% in *N. muscorum* and by 39, 64, and 54% in *Anabaena* sp. at 50, 100, and 150 mM of As^V, respectively, after 48 h of exposure. Similar increase in DHAR activity was noticed in As^{III} treated cells of both the cyanobacteria. Moreover, the DHAR activity was further raised after 96 h of treatment at lower doses of As^V (50 and 100 mM) and As^{III} (50 and 100 μM), while lesser increase was recorded at higher doses of As^V (150 mM) and As^{III} (150 μM), but still greater than control.

Glutathione reductase (GR) activity and its isoenzyme patterning

The results pertaining to GR activity in test cyanobacteria under As^V and As^{III} exposure have been depicted in Fig. 3c. Results revealed that after 48 h of treatment, As^V at 50, 100, and 150 mM dose raised the GR activity by 56, 83, and 71% in *N. muscorum* and by 50, 74, and 63% in *Anabaena* sp.,



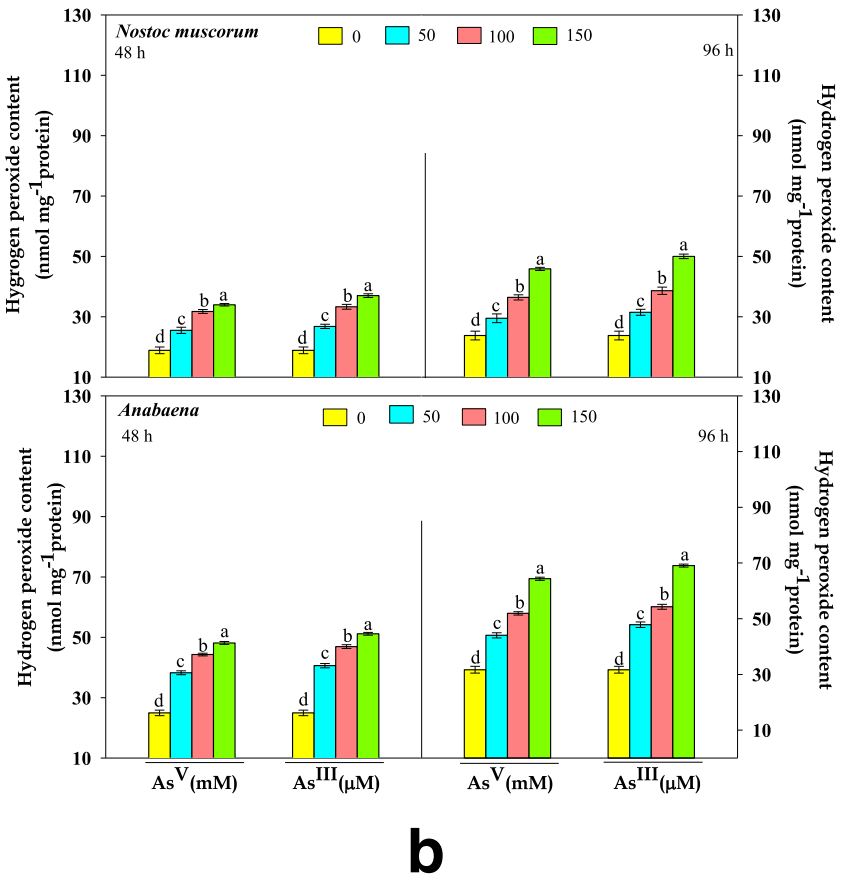


Fig. 2 Effect of arsenate (As^{V}) and arsenite (As^{III}) on oxidative stress biomarkers (SOR (**a**), H_2O_2 (**b**), and MDA equivalents content (**c**)) of *Nostoc muscorum* ATCC 27893 and *Anabaena* PCC 7120 after 48 h and 96 h of treatments. Data are means ± standard error of the three replicates ($n = 3$). Bars followed by different letters show significant difference at $P < 0.05$ according to the Duncan multiple range test (DMRT)

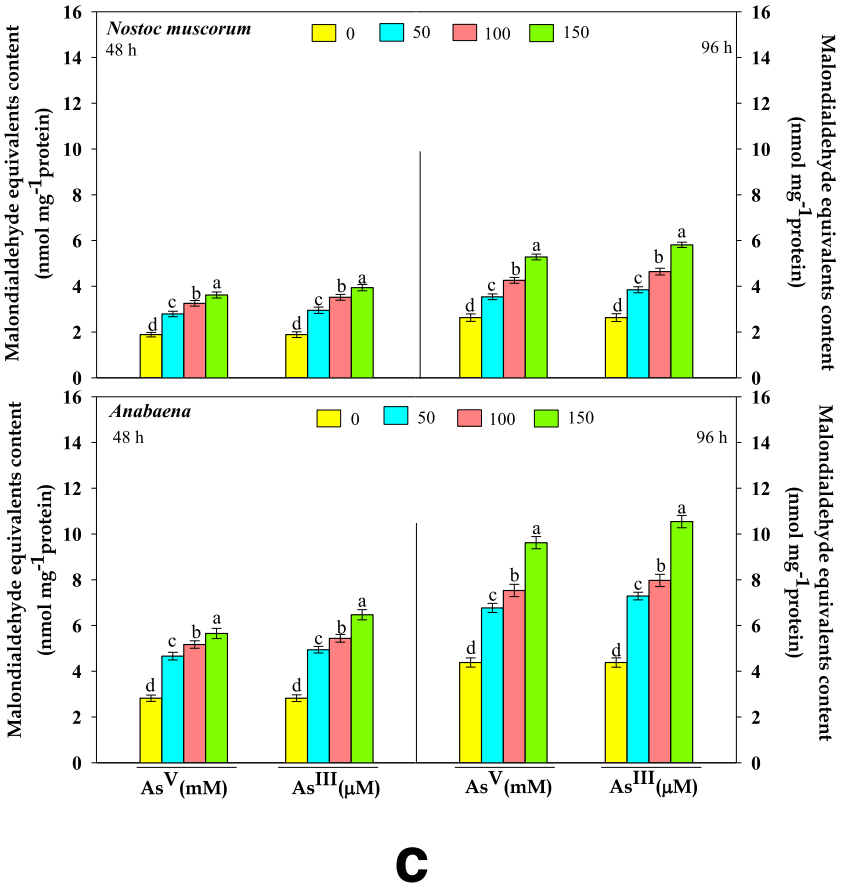


Fig. 2 continued.

respectively, over the values of control. Similarly As^{III} enhanced the GR activity in both the test cyanobacteria. Moreover, after 96 h of treatment, both As^{V} and As^{III} further raised GR activity at lower doses of As^{V} (50 and 100 mM) and As^{III} (50 and 100 μM), while lesser increase was noticed at higher doses of As^{V} (150 mM) and As^{III} (150 μM), but still greater than control.

The results related to GR isoenzyme have been portrayed in Fig. 3d. Native–PAGE gel showed single isoform of GR, i.e., GR I in both the cyanobacteria. However, the band intensity of the isoform showed differential expression in both the organism. Overall results suggested that As^{V} and As^{III} treatment raised the APX, DHAR, and GR activities but the increase was more pronounced in *N. muscorum*.

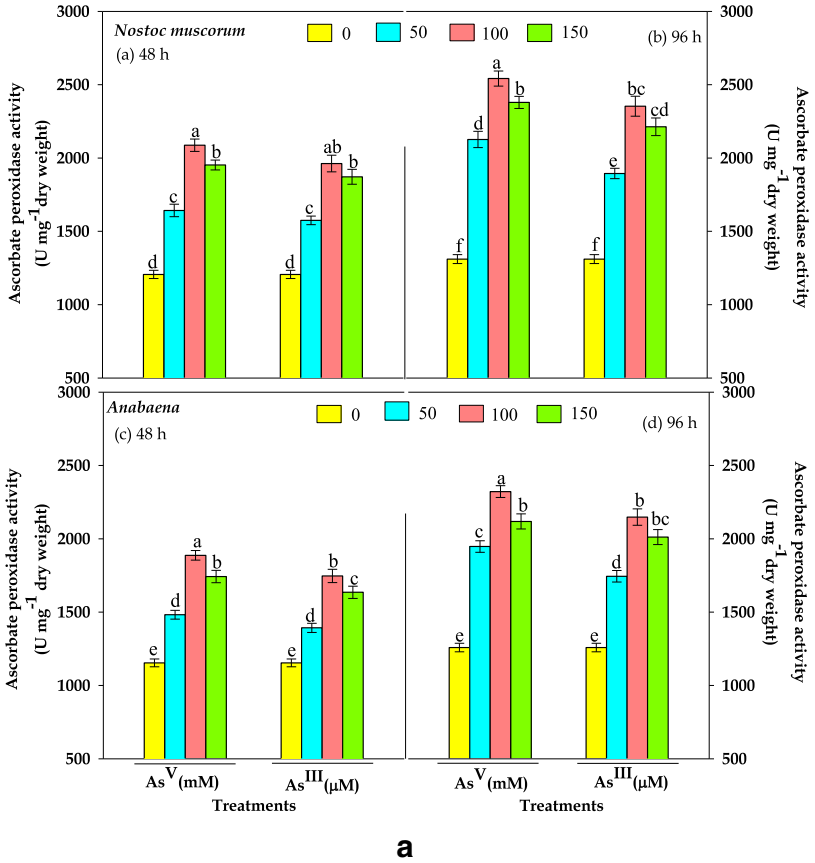
(B) Metabolites of ascorbate-glutathione cycle (AsA-GSH) cycle

Reduced (AsA) and oxidized (DHA) ascorbate

The impact on reduced ascorbate (AsA), oxidized ascorbate, i.e., dehydroascorbate (DHA), and AsA/DHA was investigated in *N. muscorum* and *Anabaena* sp., following As^{V} and As^{III} exposure has been shown in Table 2. Results revealed that As^{V} and As^{III} treatment at all the doses declined the AsA content while enhanced DHA content in both the test cyanobacteria. This disturbance to AsA and DHA pool declined the AsA/DHA ratio and this ratio was found to show declining trend with increasing As^{V} and As^{III} doses after 48 h of treatment. Moreover, after 96 h of treatment, similar decline in AsA content and AsA to DHA ratio, while increase in DHA content was observed in both the test cyanobacteria treated with As^{V} and As^{III}. As compared to *Anabaena* sp., *N. muscorum* cells showed greater values of AsA and AsA to DHA ratio that suggests the reason for resistant nature of *Nostoc* under test conditions.

Reduced (GSH) and oxidized (GSSG)

The impact on reduced glutathione (GSH), oxidized glutathione (GSSG), and GSH to GSSG ratio was investigated in *N. muscorum* and *Anabaena* sp. following As^{V} and As^{III} exposure and results have been shown in Table 3. Results revealed that As^{V} and As^{III} treatment at all the doses declined the GSH content while enhanced GSSG content in both the



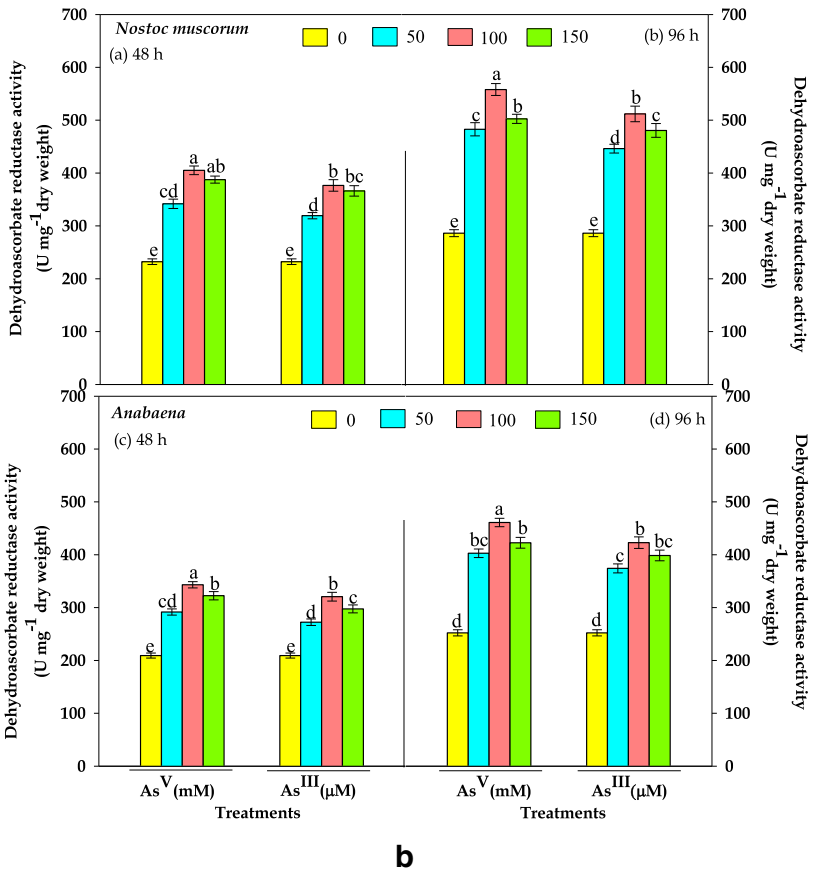
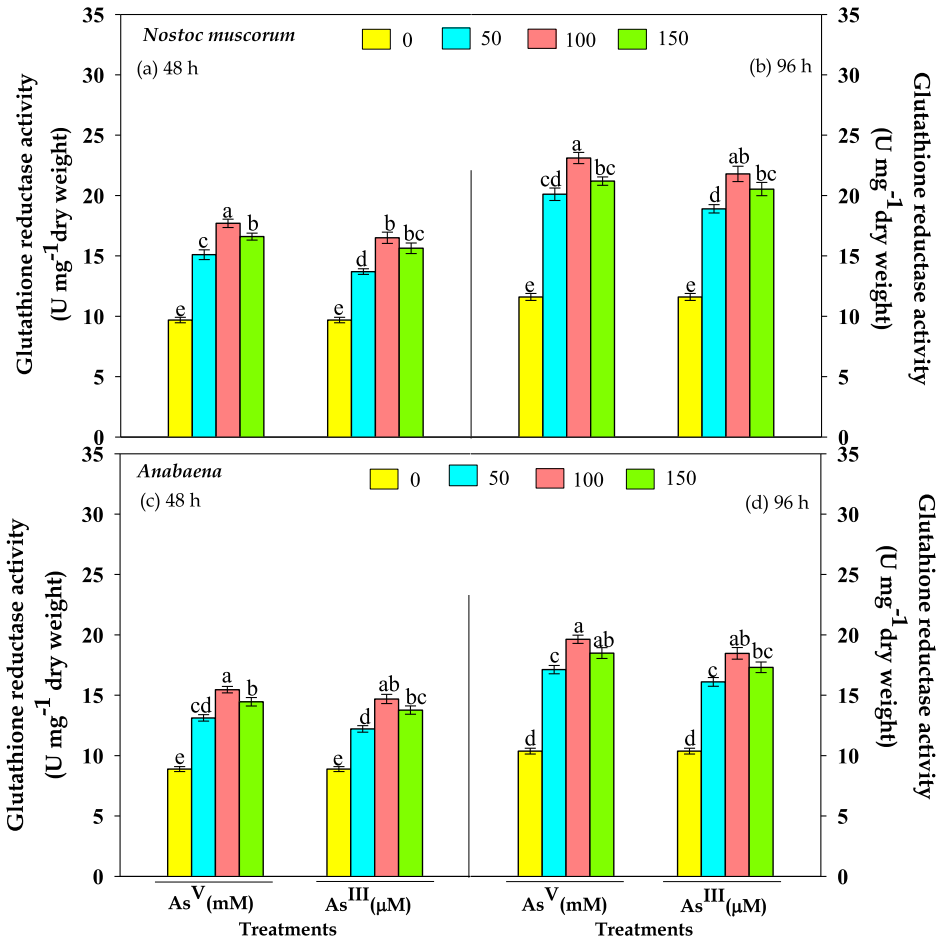


Fig. 3 **a** Effect of arsenate (As^V) and arsenite (As^{III}) on ascorbate peroxidase (APX) activity in *Nostoc muscorum* ATCC 27893 and *Anabaena* PCC 7120 after 48 h and 96 h of treatments. Data are means ± standard error of the three replicates ($n = 3$). Bars followed by different letters show significant difference at $P < 0.05$ according to the Duncan multiple range test (DMRT). **b** Effect of arsenate (As^V) and arsenite (As^{III}) on dehydroascorbate reductase (DHAR) activity in *Nostoc muscorum* ATCC 27893 and *Anabaena* PCC 7120 after 48 h and 96 h of treatments. Data are means ± standard error of the three replicates ($n = 3$). Bars followed by different letters show significant difference at $P < 0.05$ according to the Duncan multiple range test (DMRT). **c** Effect of arsenate (As^V) and arsenite (As^{III}) on glutathione reductase (GR) activity in *Nostoc muscorum* ATCC 27893 and *Anabaena* PCC 7120 after 48 h and 96 h of treatments. Data are means ± standard error of the three replicates ($n = 3$). Bars followed by different letters show significant difference at $P < 0.05$ according to the Duncan multiple range test (DMRT). **d** Effect of arsenate (As^V) and arsenite (As^{III}) on isoenzyme profiling (native-PAGE) of ascorbate peroxidase (APX) and glutathione reductase (GR) in *Nostoc muscorum* ATCC 27893 (**a**) and *Anabaena* PCC 7120 (**b**). Lanes represent **1**: Control, **2**: 50 mM As^V, **3**: 100 mM As^V, **4**: 150 mM As^V, **5**: 50 μM As^{III}, **6**: 100 μM As^{III}, **7**: 150 μM As^{III}. (Arrow indicates the isoforms of enzymes)



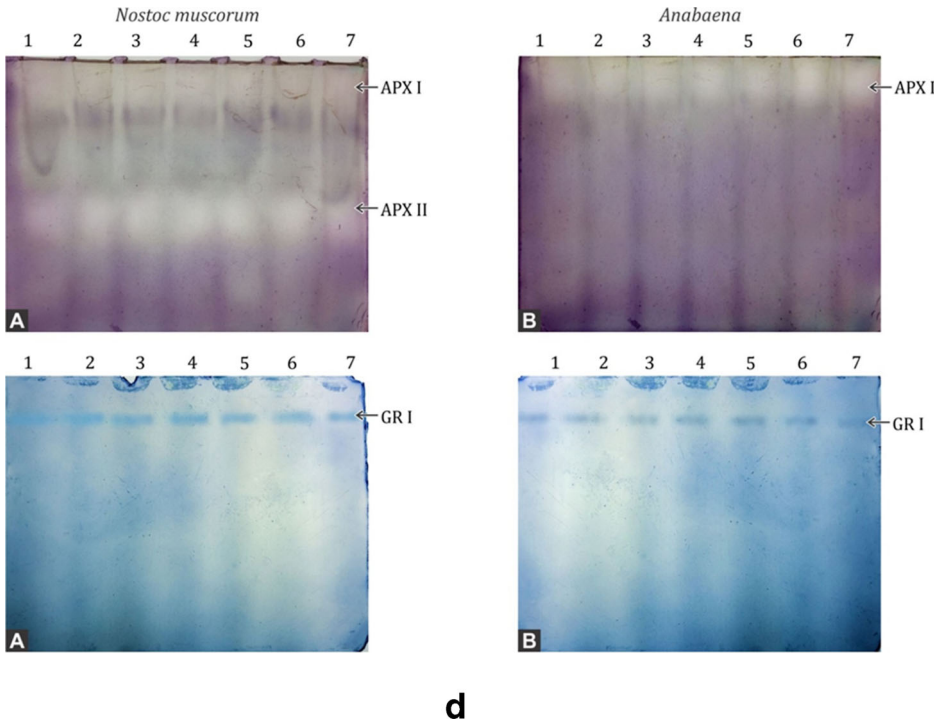


Fig. 3 continued.

Table 2 Effect of arsenate (As^V) and arsenite (As^{III}) on the contents of reduced ascorbate (AsA), dehydroascorbate (DHA), and ratios of AsA to DHA and AsA to H_2O_2 of *Nostoc muscorum* ATCC 27893 and *Anabaena* PCC 7120 after 48 h and 96 h of treatments

Treatment *Nostoc muscorum*		Antioxidant contents (μmol g^{-1} dry weight)				Ratios			
		Reduced ascorbate (AsA)		Dehydroascorbate (DHA)		AsA/DHA		AsA/H_2O_2	
		48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h
Control	0	61.20 ± 1.38^{a}	86.30 ± 2.02^{a}	8.20 ± 0.17^{f}	10.60 ± 0.23^{f}	7.48 ± 0.17^{a}	8.14 ± 0.20^{a}	8.86 ± 0.20^{a}	7.69 ± 0.17^{a}
Arsenate As^V (mM)	50	54.40 ± 1.38^{b}	78.70 ± 2.02^{b}	10.20 ± 0.28^{e}	12.30 ± 0.34^{e}	5.34 ± 0.14^{b}	6.39 ± 0.17^{b}	4.90 ± 0.11^{b}	4.49 ± 0.11^{b}
	100	44.39 ± 0.92^{c}	67.60 ± 1.38^{c}	11.30 ± 0.23^{cd}	13.60 ± 0.28^{d}	3.94 ± 0.08^{d}	4.95 ± 0.08^{d}	3.60 ± 0.05^{d}	3.19 ± 0.05^{d}
	150	35.90 ± 0.63^{d}	47.60 ± 0.80^{d}	11.80 ± 0.23^{bc}	16.50 ± 0.28^{b}	3.04 ± 0.28^{f}	2.89 ± 0.05^{f}	2.45 ± 0.02^{f}	1.95 ± 0.02^{e}
Arsenite As^{III} (μM)	50	52.60 ± 0.98^{b}	76.60 ± 1.44^{b}	10.70 ± 0.17^{de}	13.10 ± 0.23^{de}	$0.08^{c}4.93 \pm$	5.84 ± 0.08^{c}	4.54 ± 0.08^{c}	4.13 ± 0.08^{c}
	100	42.10 ± 1.12^{c}	64.59 ± 1.84^{c}	12.40 ± 0.34^{b}	15.30 ± 0.46^{c}	3.39 ± 0.11^{e}	4.20 ± 0.11^{e}	2.86 ± 0.08^{e}	2.94 ± 0.08^{d}
	150	33.36 ± 0.92^{d}	43.80 ± 1.18^{d}	13.63 ± 0.37^{a}	18.63 ± 0.49^{a}	2.44 ± 0.08^{g}	2.34 ± 0.08^{g}	2.10 ± 0.05^{g}	1.65 ± 0.02^{f}
Anabaena PCC 7120									
Control	0	55.70 ± 1.28^{a}	81.10 ± 1.87^{a}	8.90 ± 0.20^{f}	11.20 ± 0.25^{f}	6.27 ± 0.14^{a}	7.22 ± 0.16^{a}	6.88 ± 0.15^{a}	6.82 ± 0.15^{a}
Arsenate As^V (mM)	50	46.90 ± 0.94^{b}	71.20 ± 1.43^{b}	12.10 ± 0.24^{e}	14.10 ± 0.28^{e}	3.87 ± 0.07^{b}	5.04 ± 0.10^{b}	4.61 ± 0.09^{b}	3.69 ± 0.07^{b}
	100	38.10 ± 0.65^{c}	59.70 ± 1.03^{c}	13.40 ± 0.23^{cd}	15.90 ± 0.27^{cd}	2.84 ± 0.04^{d}	3.75 ± 0.06^{d}	3.10 ± 0.05^{d}	2.68 ± 0.04^{d}
	150	29.50 ± 0.71^{d}	38.40 ± 0.93^{d}	14.50 ± 0.35^{b}	19.30 ± 0.46^{b}	2.03 ± 0.36^{f}	1.98 ± 0.04^{f}	2.21 ± 0.05^{f}	1.49 ± 0.03^{f}
Arsenite As^{III} (μM)	50	44.90 ± 1.01^{b}	68.40 ± 1.54^{b}	12.90 ± 0.29^{de}	14.90 ± 0.33^{de}	3.48 ± 0.07^{e}	4.59 ± 0.10^{c}	3.91 ± 0.08^{c}	3.20 ± 0.07^{c}
	100	35.50 ± 0.92^{c}	56.30 ± 1.46^{c}	14.20 ± 0.36^{bc}	16.92 ± 0.43^{c}	2.50 ± 0.06^{c}	3.32 ± 0.08^{e}	2.73 ± 0.07^{e}	2.33 ± 0.06^{e}
	150	27.10 ± 0.68^{d}	34.60 ± 0.87^{d}	15.80 ± 0.40^{a}	20.70 ± 0.52^{a}	1.71 ± 0.43^{g}	1.67 ± 0.42^{g}	1.79 ± 0.04^{g}	1.19 ± 0.03^{g}

Data are means± standard error of the three replicates ($n = 3$). Values followed by different letters show significant difference at $P < 0.05$ according to the Duncan multiple range test (DMRT)

Table 3 Effect of arsenate (As^V) and arsenite (As^{III}) on the contents of reduced glutathione (GSH), oxidized glutathione (GSSG), and ratios of GSH to GSSG of *Nostoc muscorum* ATCC 27893 and *Anabaena* PCC 7120 after 48 h and 96 h of treatments

Treatment *Nostoc muscorum*		Antioxidant contents (nmol g^{-1} dry weight)				Ratio	
		Reduced glutathione (GSH)		Oxidized glutathione (GSSG)		GSH/GSSG	
		48 h	96 h	48 h	96 h	48 h	96 h
Control	0	29.60 ± 0.69^{a}	48.55 ± 1.12^{a}	3.90 ± 0.11^{f}	6.14 ± 0.14^{e}	7.60 ± 0.17^{a}	7.94 ± 0.20^{a}
Arsenate As^V (mM)	50	26.90 ± 0.69^{b}	45.20 ± 1.15^{b}	4.70 ± 0.11^{e}	6.99 ± 0.17^{d}	5.69 ± 0.17^{b}	6.49 ± 0.17^{b}
	100	20.90 ± 0.40^{c}	36.10 ± 0.75^{c}	5.60 ± 0.11^{c}	8.30 ± 0.17^{c}	3.74 ± 0.08^{d}	4.34 ± 0.08^{d}
	150	16.90 ± 0.28^{d}	25.10 ± 0.46^{d}	6.10 ± 0.10^{b}	10.30 ± 0.17^{b}	2.79 ± 0.05^{f}	2.44 ± 0.02^{f}
Arsenite As^{III} (μM)	50	25.90 ± 0.46^{b}	44.10 ± 0.80^{b}	5.14 ± 0.08^{d}	7.55 ± 0.14^{d}	5.04 ± 0.08^{c}	5.84 ± 0.08^{c}
	100	19.60 ± 0.57^{c}	34.34 ± 1.01^{c}	5.90 ± 0.17^{bc}	8.75 ± 0.25^{c}	3.34 ± 0.08^{e}	3.90 ± 0.11^{e}
	150	15.33 ± 0.43^{d}	22.53 ± 0.60^{d}	6.50 ± 0.17^{a}	11.0 ± 0.31^{a}	2.38 ± 0.05^{g}	2.04 ± 0.02^{g}
Anabaena PCC 7120							
Control	0	27.80 ± 0.64^{a}	44.62 ± 1.03^{a}	4.50 ± 0.10^{e}	6.30 ± 0.14^{e}	6.18 ± 0.14^{a}	7.09 ± 0.16^{a}
Arsenate As^V (mM)	50	24.10 ± 0.48^{b}	40.30 ± 0.81^{b}	5.90 ± 0.11^{d}	7.80 ± 0.15^{d}	4.08 ± 0.08^{b}	5.16 ± 0.10^{b}
	100	18.50 ± 0.32^{c}	31.40 ± 0.54^{c}	6.80 ± 0.11^{c}	9.10 ± 0.15^{c}	2.72 ± 0.04^{d}	3.45 ± 0.05^{d}
	150	14.40 ± 0.34^{e}	19.90 ± 0.48^{d}	7.60 ± 0.18^{b}	11.30 ± 0.27^{b}	1.89 ± 0.04^{f}	1.76 ± 0.04^{f}
Arsenite As^{III} (μM)	50	22.80 ± 0.51^{b}	39.20 ± 0.88^{b}	6.40 ± 0.14^{c}	8.40 ± 0.18^{b}	3.56 ± 0.08^{e}	4.66 ± 0.10^{c}
	100	16.90 ± 0.43^{d}	29.30 ± 0.76^{c}	7.30 ± 0.18^{b}	9.70 ± 0.25^{c}	2.31 ± 0.06^{c}	3.02 ± 0.07^{e}
	150	12.60 ± 0.32^{f}	18.30 ± 0.46^{d}	8.20 ± 0.20^{a}	12.20 ± 0.30^{a}	1.53 ± 0.03^{g}	1.50 ± 0.03^{f}

Data are means ± standard error of the three replicates ($n = 3$). Values followed by different letters show significant difference at $P < 0.05$ according to the Duncan multiple range test (DMRT)

test cyanobacteria. This disturbance to GSH and GSSG declines the GSH/GSSG ratio and this ratio was found to show declining trend with increasing As^V and As^{III} doses after 48 h of treatment. Moreover, after 96 h of treatment similar decline in GSH content and GSH to GSSG ratio, while increase in GSSG content was observed in both the test cyanobacteria treated with As^V and As^{III}. Further, *N. muscorum* cells showed greater values of GSH and GSH to GSSG ratio than *Anabaena* sp. that suggests the reason for resistant nature of *Nostoc* under test conditions.

Non-enzymatic antioxidants

Cysteine (Cys) content

Results pertaining to cysteine content in As^V and As^{III} stressed cyanobacteria after 48 and 96 h of treatment have been depicted in Table 4. After 48 h of treatment, results showed that exposure of *N. muscorum* to As^V at 50, 100, and 150 mM raised the content by 38, 83, and 71% and by 28, 73, and 62% at 50, 100, and 150 μM of As^{III}, respectively, over the control values. Under similar condition, *Anabaena* sp. treated cells showed an enhancement of only 31, 76, and 62% at 50, 100, and 150 mM of As^V exposure and by 21, 62, and 54%, at 50, 100, and 150 μM of As^{III}, respectively. After 96 h of treatment, both the test cyanobacteria under As^V and As^{III} exposure showed further enhancement in Cys content at all the test doses and the values recorded were greater in *N. muscorum* than *Anabaena* sp. showing its resistant nature against As stress.

Proline (Pro) content

The results pertaining to proline content in test cyanobacteria under As^V and As^{III} stress after 48 and 96 h of treatment have been presented in Table 4. The Pro content was found to be increased by 48, 96, and 76% in *N. muscorum* at 50, 100, and 150 mM of As^V and by 41, 86, and 72% in *Anabaena* sp., respectively, over the control values after 48 h of treatment. Moreover, the increment in Pro content was recorded by 41, 83, and 73% in *N. muscorum* and by 34, 79, and 65% in *Anabaena* sp. at 50, 100, and 150 μM of As^{III}, respectively, after 48 h of treatment. Similarly, after 96 h of treatment, further increase in Pro content was recorded at all the test doses.

Non-protein thiol (NP-SH) content

Results pertaining to NP-SH content in test cyanobacteria in response to As^V and As^{III} stress have been depicted in Table 4. After 48 h of treatment, As^V at 50, 100, and 150 mM raised the content by 40, 88, and 81% in *N. muscorum* and by 32, 81,

Table 4 Effect of arsenate (As^V) and arsenite (As^{III}) on the non-enzymatic antioxidants: proline (Pro), cysteine (Cys), non-protein thiols (NP-SH), total phenolic content (TPC), and phytochelatins (PCs) of *Nostoc muscorum* ATCC 27893 and *Anabaena* PCC 7120 after 48 h and 96 h of treatments

		Non-enzymatic antioxidants							
Treatment *Nostoc muscorum*		Proline (Pro) [nmol (mg^{-1} dry weight)]		Cysteine (Cys) [nmol (mg^{-1} dry weight)]		Non-protein thiols (NP-SH) [nmol (mg^{-1} dry weight)]		Total phenolic content (TPC) [μg GAE (mg^{-1} dry weight)]	
		48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h
Control	0	6.26 ± 0.14^{d}	9.03 ± 0.20^{f}	3.09 ± 0.05^{e}	4.64 ± 0.08^{d}	152 ± 3.52^{d}	181 ± 4.18^{d}	3.66 ± 0.08^{e}	4.90 ± 0.11^{d}
Arsenate As^V (mM)	50	9.30 ± 0.23^{c}	16.20 ± 0.40^{de}	4.25 ± 0.08^{d}	7.44 ± 0.20^{c}	213 ± 5.57^{c}	297 ± 7.76^{c}	5.73 ± 0.14^{cd}	8.56 ± 0.20^{c}
	100	12.30 ± 0.23^{a}	19.43 ± 0.37^{a}	5.61 ± 0.11^{a}	9.29 ± 0.17^{a}	287 ± 5.80^{a}	378 ± 7.64^{a}	6.66 ± 0.14^{a}	9.93 ± 0.20^{a}
	150	11.00 ± 0.20^{b}	17.40 ± 0.28^{bc}	5.25 ± 0.08^{bc}	8.56 ± 0.14^{b}	276 ± 4.79^{a}	358 ± 6.20^{a}	6.40 ± 0.11^{ab}	9.30 ± 0.17^{ab}
Arsenite As^{III} (mM)	50	8.86 ± 0.14^{c}	15.40 ± 0.28^{e}	3.94 ± 0.08^{d}	6.95 ± 0.14^{c}	207 ± 3.81^{c}	291 ± 5.39^{c}	5.50 ± 0.11^{d}	8.16 ± 0.14^{c}
	100	11.50 ± 0.34^{b}	18.00 ± 0.51^{b}	5.34 ± 0.14^{ab}	8.80 ± 0.23^{ab}	272 ± 7.88^{ab}	356 ± 10.30^{a}	6.30 ± 0.17^{ab}	9.50 ± 0.28^{a}
	150	11.90 ± 0.28^{b}	16.73 ± 0.43^{cd}	4.99 ± 0.11^{c}	8.29 ± 0.23^{b}	256 ± 6.96^{b}	326 ± 8.81^{b}	6.10 ± 0.17^{bc}	8.70 ± 0.20^{bc}
Anabaena PCC 7120									
Control	0	5.27 ± 0.12^{e}	8.12 ± 0.18^{f}	2.37 ± 0.05^{d}	3.97 ± 0.09^{d}	141 ± 3.26^{a}	169 ± 3.91^{e}	2.68 ± 0.06^{d}	3.98 ± 0.09^{e}
Arsenate As^V (mM)	50	7.40 ± 0.14^{d}	13.96 ± 0.28^{de}	3.09 ± 0.06^{c}	6.97 ± 0.14^{c}	187 ± 3.78^{c}	269 ± 5.45^{d}	3.92 ± 0.07^{c}	6.57 ± 0.13^{cd}
	100	9.78 ± 0.16^{a}	16.41 ± 0.28^{a}	4.15 ± 0.07^{a}	8.72 ± 0.15^{a}	256 ± 4.46^{a}	336 ± 5.83^{a}	4.55 ± 0.07^{a}	7.62 ± 0.13^{a}
	150	9.04 ± 0.21^{bc}	15.36 ± 0.37^{b}	3.82 ± 0.09^{b}	8.04 ± 0.19^{b}	241 ± 5.84^{ab}	309 ± 7.50^{b}	4.25 ± 0.10^{b}	7.04 ± 0.17^{bc}
Arsenite As^{III} (mM)	50	7.03 ± 0.15^{d}	13.14 ± 0.29^{e}	2.85 ± 0.06^{c}	6.58 ± 0.14^{c}	177 ± 3.98^{c}	250 ± 5.64^{d}	3.74 ± 0.08^{c}	6.36 ± 0.14^{d}
	100	9.43 ± 0.24^{ab}	15.08 ± 0.39^{bc}	3.80 ± 0.09^{b}	8.20 ± 0.21^{b}	241 ± 6.27^{ab}	317 ± 8.24^{ab}	4.28 ± 0.11^{ab}	7.25 ± 0.18^{ab}
	150	8.66 ± 0.22^{c}	14.17 ± 0.36^{cd}	3.62 ± 0.09^{b}	7.70 ± 0.19^{b}	226 ± 5.74^{b}	289 ± 8.81^{c}	4.01 ± 0.10^{bc}	6.65 ± 0.16^{cd}

Data are means ± standard error of the three replicates ($n = 3$). Values followed by different letters show significant difference at $P < 0.05$ according to the Duncan multiple range test (DMRT)

and 70% in *Anabaena* sp., respectively. The As^{III} at 50, 100, and 150 μM raised the content by 35, 78, and 67% in *N. muscorum* and by 25, 70, and 59% in *Anabaena*, respectively. Moreover after 96 of treatment, further enhancement in NP-SH content was recorded at all the test doses of As^{V} and As^{III} and the values recorded were higher in *N. muscorum* than *Anabaena* sp.

Total phenolic (TPs) content

The results for total phenolic contents in test cyanobacteria under As^{V} and As^{III} exposure have been depicted in Table 4. Results revealed that As^{V} at 50, 100, and 150 mM significantly enhanced the TPs content by 56, 82, and 74% in *N. muscorum* and by 47, 70, and 59% in *Anabaena* sp., respectively, over the control value. Similar to As^{V}, As^{III} at 50, 100, and 150 μM raised the content by 49, 72, and 65% in *N. muscorum* and 40, 60, and 50% in *Anabaena* sp. after 48 h of treatment. Moreover after 96 h of treatment, further increment in TPs content was recorded at all the tested doses.

Discussion

Heavy metal toxicity is a serious threat as it significantly affects the growth and development associated with hampered physiological and biochemical processes via inducing oxidative stress of photosynthetic organisms, including cyanobacteria (Hurtado-Gallego et al. 2018; Patel et al. 2018). In the present study, two redox forms of As, i.e., As^{V} and As^{III} caused deleterious impact on growth of *Nostoc muscorum* ATCC 27893 and *Anabaena* sp. PCC 7120 and the tolerance behavior of tested cyanobacteria is marginally increased after increasing the successive time interval, i.e., from 48 to 96 h. After 48 h, reduction in growth (measured in terms of RGR) (Fig. 1a) and increased SI (Fig. 1b) was noticed under tested doses of both As^{V} and As^{III}. Decrease in growth is due to increase As bio-accumulation (Table 1), oxidative stress biomarkers (Fig. 2), and reduced efficiency of H_2O_2 detoxification cycle, i.e., AsA-GSH cycle (Fig. 3). Earlier reports of Upadhyay et al. (2016) and Patel et al. (2018) described the similar reason for decrease in the growth of *Nannochloropsis* and *Nostoc*. However, when the tested cyanobacteria kept under similar conditions for longer duration (96 h), a marginal recovery in growth was observed with lower doses, i.e., 50 and 100 mM for As^{V}; 50 and 100 μM for As^{III}. The possible reason behind minimizing the As^{V}/As^{III}-induced toxicity is due to fact that cyanobacteria have the inherent ability to convert toxic forms of As into less toxic forms and also excrete the As via the process of biomethylation and volatilization (Ye et al. 2012). This is the fact behind tolerance and bioremediant behavior of cyanobacteria exposed to higher concentration of As for longer durations (Ye et al. 2012). Upon comparing the sensitivity index, it was found that the *Anabaena* sp. showed more sensitivity (Fig. 1b) against As than that of *N. muscorum* is might be due to absence of gelatinous sheath as present in *Nostoc*. On comparing the toxicity between two redox forms, it was found that As^{III} proves to be more toxic due to its passive transportation *via* aquaglyceroporins (AQGP) across the membrane (Miyashita et al. 2015) that leads higher intracellular As accumulation altered the protein structure by attacking -SH group to form arsenothiols Finnegan and Chenm (2012). Our results are in agreement with earlier reports of Ferrari et al. (2013) and Sure et al. (2016) and proved more toxic nature of As^{III} than As^{V}. Zutshi et al. (2014) in his study reported that the reduction in growth is associated with increased intracellular As accumulation (Table 1), oxidative stress biomarkers, and weak antioxidant machinery (Figs. 2 and 3). In our study, under 150 mM of As^{V} and 150 μM of As^{III} tested cyanobacteria showed only 50% growth reduction, suggesting that cyanobacteria could tolerate such higher concentration of As and behaves as bio-accumulator. Further, time-dependent recovery increases the tolerance behavior of cyanobacteria against As toxicity, as evident that after 96 h, lower doses (50 and 100 mM of As^{V} and 50 and 100 μM of As^{III}) reflect slight improvement in growth of both the cyanobacteria as the degree of toxicity was lesser than 48 h.

Arsenic is well known to induce oxidative stress in photoautotrophs by generating reactive oxygen species (ROS) such as superoxide radicle SOR ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) and organism is in oxidative stress. Severe oxidative stress subsequently damaged the membrane lipids or increased the lipid peroxidation (measured in terms of malondialdehyde (MDA) equivalent contents) (Itri et al. 2014; Farooq et al. 2015) that caused inactivation of enzymes or membrane-bound receptors. The present study showed that *N. muscorum* and *Anabaena* sp. under As^{V}/As^{III} stress exhibited high levels of ROS ($O_2^{\cdot-}$ and H_2O_2) associated with increased MDA equivalent content (Fig. 2 a, b, and c). Excessive generation of ROS under As stress is might be due to leakage of electrons during reduction of molecular oxygen at the end of non-cyclic photosynthetic electron transport chain (Zhao et al. 2007). Further, these ROS rapidly diffused in cytosol and damaged the macro-molecules (protein, lipids, and nucleic acids) associated with reduction in growth (Singh et al., 2016a, b; Prajapati et al. 2018). Our results are in synchronization with earlier findings where As^{V}/As^{III} was described to cause oxidative stress in *Haplosiphon* (Zutshi et al. 2014) and in *Anabaena* PCC 7120 (Pandey et al. 2013). Due to high permeability and stability across the plasma membrane and have capability to generate hydroxyl radicals ($^{\cdot}OH$) by reacting with Fe^{2+} or Cu^{2+}, H_2O_2 proves to be more toxic (Gill and Tuteja 2010). On comparing the toxicity of *N. muscorum* and *Anabaena* sp. on exposure to metal, *Anabaena* sp. was more prone against As toxicity as evident

by increased ROS generation and feeble antioxidant machinery.

To cope with the damaging effects induced by ROS, photosynthetic organisms have an array of antioxidant defense mechanisms (enzymatic as well as non-enzymatic) that are good indicator of internal cell situation. In this series, ascorbate-glutathione (AsA-GSH) cycle takes charge over catalase (CAT) and peroxidases (POD) to mediated H_2O_2 detoxification that operates in cytosol. Activity of AsA-GSH cycle depends on three enzymes APX, GR, and DHAR and its metabolites: AsA and GSH that interchangeable into oxidized or reduced forms (Wu et al. 2017). Under stress conditions, efficiency of AsA-GSH cycle is a key factor that regulates the oxidative stress. In AsA-GSH cycle, APX is the key enzyme that converts H_2O_2 into H_2O and O_2 by utilizing AsA as electron donor and reducing it to monodehydroascorbate (MDHA) (Correa-Aragunde et al. 2013). In the present study, enhancement of APX activity was noticed in both the test cyanobacteria under As^V/As^{III} stress (Fig. 3a), which is concurrent with earlier findings of Piotrowska-Niczyporuk et al. (2015) and Dhuldhaja et al. (2018). Increased activity of APX minimized the quenching of oxy-radicals by decreasing the AsA pool (Smirnoff and Wheeler 2000). Under similar conditions, oxidized form of AsA, i.e., DHA was found to be increased thereby declined the AsA/DHA ratio (Table 2). Efficiency of AsA-GSH cycle depends on replenishment of AsA mediated by DHAR that uses GSH as electron donor and converts it into GSSG and a remarkable decrease in GSH pool was noticed under As stress associated with declined GSH/GSSG ratio (Table 3). Similar decrease in the metabolites of AsA-GSH cycle was noticed under As stress in *Phormidium foveolarum* (Bhattacharya and Pal 2012), and in coriander Asad ikarama et al. (2017). Reduction in GSH content under As stress is might be due to its direct involvement in phytochelatin synthesis (Mishra and Dubey 2006). Furthermore, replenishment of GSH was mediated by enzyme GR that uses NADPH as electron donor which was found to be increased in present study (Fig. 3c) thus maintains GSH/GSSG ratio Chattergee et al. (2018). In AsA-GSH cycle, GR is interconnected with APX activity in which AsA pool was refurbished by GSH and NADPH. Higher concentration of As caused severe oxidative stress as evident by decrease in the ratio of AsA/DHA, GSH/GSSG, and AsA/H_2O_2 (Tables 2 and 3). After increasing time interval, the increase in the tolerance behavior of both the tested cyanobacteria was noticed and this is best explained on the basis of status of AsA-GSH cycle. After 96 h, with the lower doses (50 and 100 mM of As^V; 50 and 100 μM of As^{III}), further increase in the efficiency of AsA-GSH cycle was noticed and also maintained the ratio of AsA/DHA, GSH/GSSG as compared to 48 h indicating that cyanobacteria is trying to overcome the oxidative stress and exhibits growth improvement.

In addition to enzymatic antioxidant, non-enzymatic antioxidants are naturally present and behave as direct quencher of ROS. In the present study, As^V and As^{III} significantly enhanced the contents of non-enzymatic antioxidants such as cysteine (Cys), proline (Pro), total phenolic content (TPC), and non-protein thiols (NP-SH) in dose-dependent manner (Table 4). In addition to this, chelation of toxic metalloids (As) in cytosol was mediated by the combined actions of –SH containing antioxidants such as Cys or NP-SH that reduces the extent of oxidative stress (Banerjee et al. 2017). Similar results were also observed by Awasthi et al. (2018) under As stress that describes the direct role of Cys in GSH and phytochelatins (PCs) bio-synthesis. Phytochelatins (PCs) also play an essential role in withstanding the toxic effects induced by As or other heavy metals (Begum et al. 2016). Further, Cys is also involved in synthesis of metallothionein; MTs (metal binding protein) coded by gene *SmtA* and upregulation of MTs expression is a common phenomenon of cyanobacteria under heavy metal stress (Cavet et al. 2003). Hence, increase in thiol compounds makes cyanobacteria tolerant against As stress and this increase was more pronounced in *N. muscorum* as compared to *Anabaena* sp. Moreover, increase in the Pro under As stress makes cyanobacteria to withstand against osmotic stress (Aslam et al. 2017). Proline directly mediates the quenching of free radicals (Jain et al. 2001) and also possesses inherent antioxidant property (Yadav et al. 2016). In study of Singh et al. (2015), exogenous Pro significantly alleviated the As^V-induced toxicity in *Solanum melongena* that demonstrates the role of Pro as an alleviating agent. Besides this, cyanobacteria are the potential source of phenolics compounds that possess inherent antioxidant property (Chac´on-Lee and Gonz´alez-Marino 2010; Demay et al. 2019). In present study, a significant increase in TPC was noticed in both the test cyanobacteria under As^V/As^{III} stress that suggests its protective role. Our results are in agreement with the Srivastava and Sharma (2012), where As^V was found to enhance the polyphenolic contents in *Vigna mungo*.

Moreover, observed parameters were significantly affected more under As^{III} exposure than As^V which could be the obvious reason for higher damage to the cells under As^{III} treatment. Further, the resistant nature of *N. muscorum* in comparison to *Anabaena* sp. against tested stress may be attributed to lesser accumulation of ROS and higher inherent level of enzymes and metabolites of antioxidant system which are cumulatively translated into biomass. Furthermore, a hypothetical mechanism of toxicity induced by both forms of As at different levels and its time-dependent removal via the process of bio-methylation and volatilization involving arsenate reductase has been represented in Fig. 4.

Table 1S shows correlation between effect of As^V and As^{III} treatment on the studied parameters in *N. muscorum* and *Anabaena*. The data revealed that all the parameters were significantly affected by As^V and As^{III} treatment. Growth, AsA-DHA, AsA/DHA, AsA/H_2O_2, GSH, GSH/GSSG, and enzymes of AsA-GSH cycle (APX, DHAR, and GR) and non-enzymatic antioxidant (PRO, CYS, NP–SH) were found

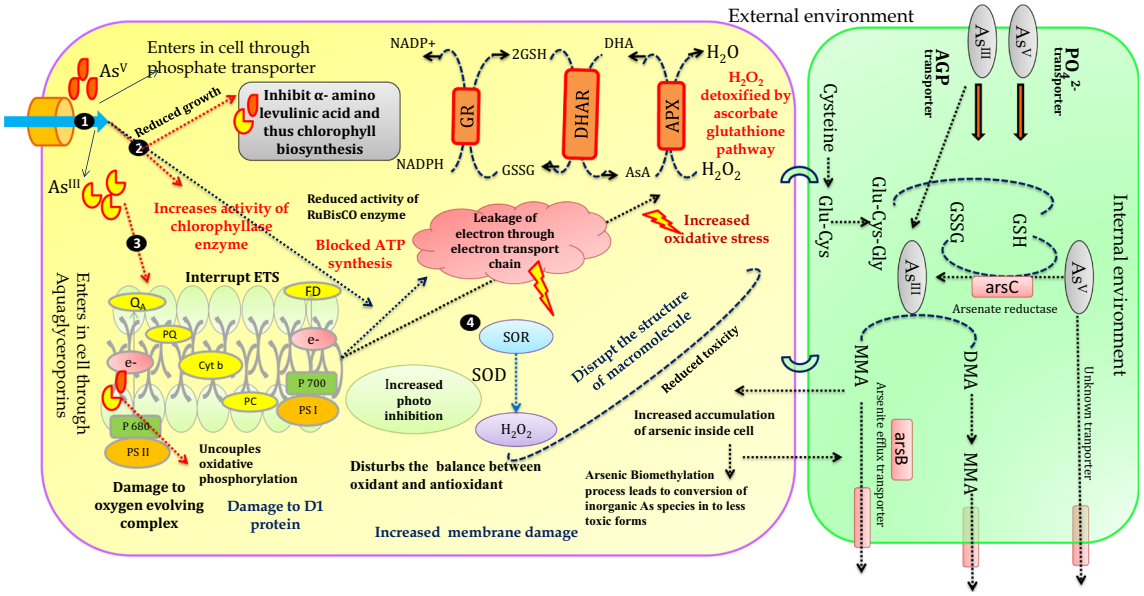


Fig. 4 Schematic representation of toxicity mediated by arsenic and its removal by bio-methylation

to be positively correlated. The value of correlation coefficient (r) suggests that effect of arsenite is more intense than As^V. A positive correlation indicates how those variables increase or decrease in parallel with the stress condition; however, a negative correlation indicates the extent to which one variable increases as the other decreases.

Conclusion

Current study proposes the deleterious impact of both the forms of arsenic, i.e., As^V and As^{III} on cyanobacteria *N. muscorum* and *Anabaena* sp. but the toxicity was overcome by time-dependent recovery. Both the stress caused damaging impact on growth due to excessive As accumulation and increase ROS content despite acceleration/enhancement in the enzymes and metabolites of AsA-GSH cycle. The results further propose recovery (but still less than control) in tested parameters at lower doses of As^V and As^{III} after 96 h of treatment. Further, the study shows that As^{III} appears to be more toxic as it declines the growth and metabolites of AsA-GSH cycle (AsA and GSH) and was noted at nearly thousand times less concentration of As^{III} (50, 100, and 150 μM) as compared to As^V (50, 100, and 150 mM). Further, the resistant nature of *N. muscorum* in comparison to *Anabaena* against As stress attributed to lesser accumulation of ROS and higher inherent level of different enzymes and metabolites of antioxidant system, which are cumulatively translated into biomass. The survival of cyanobacteria even after such high As accumulation in the cells indicates towards the potent role of these cyanobacteria as bio-fertilizers in aquatic crop system, particularly paddy fields, also as bio-remediants in As-contaminated sites.

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Author contribution SMP designed the experiment, AP and ST planned and performed the experiment, AP and ST analyzed the data, and SMP, AP, and ST wrote the manuscript.

Data availability Not applicable

Declarations

Ethics approval and consent to participate In present study no human, animals or human tissues are used and not applicable.

Consent for publication Not applicable and the manuscript does not contain individual's person data.

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

References

Asad ikarama E, Batool K, Sergio S, Viviana M, Zahra A, Hossein M, Adriana B (2017) Interaction of triacontanol and arsenic on the ascorbate-glutathione cycle and their effects on the ultrastructure in *Coriandrum sativum* L. Ecotoxicol Environ Saf 144:268–274

Aslam M, Saeed MS, Sattar S, Sajad S, Sajjad M, Adnan M, Iqbal M, Sharif MT (2017) Specific role of proline against heavy metals toxicity in plants. Int J Pure App Biosci 5:27–34

Awasthi S, Chauhana R, Dwivedi S, Srivastava S, Tripathi RD (2018) A consortium of alga (*Chlorella vulgaris*) and bacterium (*Pseudomonas putida*) for amelioration of arsenic toxicity in rice: a promising and feasible approach. Environ Exp Bot 150:115–126

Banerjee M, Chakravarty D, Ballal A (2017) Molecular basis of function and the unusual antioxidant activity of a cyanobacterial cysteine desulfurase. Biochem J 474:2435–2447

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of the free proline for water stress studies. Plant Soil 39:205–207

Begum MC, Islam MS, Islam M, Amin R, Parvez MS, Kabir AH (2016) Biochemical and molecular responses underlying differential arsenic tolerance in rice (*Oryza sativa* L.). Plant Physiol Biochem 104: 266–277

Bhattacharjee H, Mukhopadhyay R, Thiyagarajan S, Rosen BP (2008) Aquaglyceroporin: ancient channel for metalloids. J Biol 7:33–35

Bhattacharya P, Pal R (2012) Scope of phycoremediation of arsenic using *Phormidium tenue* with special reference to modulation in cellular biochemistry. J Algal Biomass Utln 3:1–8

Borella J, Becker R, Lima MC, de Oliveira DSC, Braga EJB, de Oliveira ACB, do Amarante L (2019) Nitrogen source influences the antioxidative system of soybean plants under hypoxia and re-oxygenation. Sci Agric 76:51–62

Brehe JE, Burch HB (1976) Enzymatic assay for glutathione. Anal Biochem 74:189–197

Cavet JS, Borrelly GPM, Robinson NJ (2003) Zn, Cu and Co in cyanobacteria: selective control of metal availability. FEMS Microbiol Rev 27:165–181

Chac´on-Lee TL, Gonz´alez-Marino GE (2010) Microalgae for healthy food-possibilities and challenges. Compr Rev Food Sci Food Saf 9: 655–675

Chattergee P, Biswas S, Biswas AK (2018) Sodium chloride primed seeds modulate glutathione metabolism in legume cultivars under NaCl stress. Am J Plant Physiol 13:8–22

Chen J, Rosen BP (2016) Organoarsenical biotransformations by *Shewanella putrefaciens*. Environ Sci Technol 50:7956–7963

Correa-Aragunde N, Foresi N, Delledonne M, Lamattina L (2013) Auxin induces redox regulation of ascorbate peroxidase 1 activity by S-nitrosylation/denitrosylation balance resulting in changes of root growth pattern in *Arabidopsis*. J Exp Bot 64:3339–3349

Demay J, Bernard C, Reinhardt A, Marie B (2019) Natural products from cyanobacteria: focus on beneficial activities. Mar Drugs 17:320

Dhuldhaja U, Pandyab U, Singh S (2018) Anti-oxidative response of cyanobacterium *Anabaena* sp. strain PCC 7120 to arsenite (As(III)). Microbiology 87:848–856

Ellmann GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82:70–77

Elstner EF, Heupel A (1976) Inhibition of nitrite formation from hydroxylammonium chloride: a simple assay for superoxide dismutase. Anal Biochem 70:616–620

Farooq MA, Gill RA, Ali B, Wang J, Islam F, Ali S, Zhou WJ (2015) Subcellular distribution, modulation of antioxidant and stress-related genes response to arsenic in *Brassica napus* L. Ecotoxicology 25:350–366

Ferrari SG, Silvaa PG, Gonzálezb DM, Navonic JA, Silvaa HJ (2013) Arsenic tolerance of cyanobacterial strains with potential use in biotechnology. Rev Argent Microbiol 45:174–179

Finnegan PM, Chenm W (2012) Arsenic toxicity: the effects on plant metabolism. Front Plant Sci 3:182

Gaitonde MK (1967) A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. Biochem J 104:627–633

Gebel T (2000) Confounding variables in the environmental *toxicology* of arsenic. Toxicology 144:155–162

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930

Gossett DR, Millhollon EP, Cran LM (1994) Antioxidant response to NaCl stress in salt-sensitive cultivars of cotton. Crop Sci 34:706–714

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198

Hurtado-Gallego J, Martín-Betancor K, Rodea-Palomares I, Leganés F, Rosal R, Fernández-Piñas F (2018) Two novel cyanobacterial bioluminescent whole-cell bioreporters based on superoxide dismutases MnSod and FeSod to detect superoxide anion. Chemosphere 201:772–779

Itri R, Junqueira HC, Mertins O, Baptista MS (2014) Membarne changes under oxidative stress: the impact of oxidized lipids. Biophys Rev 6(1):47–61

Jain M, Mathur G, Koul S, Sarin N (2001) Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of groundnut (*Arachis hypogaea* L.). Plant Cell Rep 20:463–468

Jedynak L, Kowalska J, Kossykowska M, Golimowski M (2010) Studies on the uptake of different arsenic forms and the influence of sample pretreatment on arsenic speciation in white mustard (*Sinapis alba*). Microchem J 94:125–129

Kulp TR, Hoeft SE, Asao N, Madigan MT, Hollibaugh JT, Fisher JC, Stolz JF, Culbertson CW, Miller LG, Oremland RS (2008) Arsenic (III) fuels anoxygenic photosynthesis in hot spring biofilms from Mono Lake, California. Sci 321:967

Kumar J, Singh VP, Prasad SM (2017) An investigation on involvement of the ascorbate-glutathione cycle in modulating NaCl toxicity in two cyanobacteria photoacclimatized to different photosynthetic active radiation. Algal Res 28:70–78

Mishra S, Dubey RS (2006) Inhibition of ribonuclease and protease activities in arsenic-exposed rice seedlings: role of proline as enzyme protectant. J Plant Physiol 163:927–936

Mittler R, Zilinskas BA (1993) Detection of ascorbate peroxidase activity in native gels by inhibition of the ascorbate–dependent reduction of nitroblue tetrazolium. Anal Biochem 212:540–546

Miyashita S, Murota C, Kondo K, Fujiwara S, Tsuzuki M (2015) Arsenic metabolism in cyanobacteria. Environ Chem 13:4

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22: 867–880

Pandey S, Shrivastava AK, Singh VK, Rai R, Singh PK, Rai S, Rai LC (2013) A new arsenate reductase involved in arsenic detoxification in *Anabaena* sp. PCC7120. Funct Integr Genom 13:43–55

Patel A, Tiwari S, Prasad SM (2018) Toxicity assessment of arsenate and arsenite on growth, chlorophyll fluorescence and antioxidant machinery in *Nostoc muscorum*. Ecotoxicol Environ Saf 157:369–379

Perales-Vela HV, Pena-Castro J, Canizares-Villanueva RO (2006) Heavy metal detoxification in eukaryotic microalgae. Chemosphere 64:1–10

Piotrowska-Niczyporuk A, Bajguz A, Talarek M, Bralska M, Zambrzycka E (2015) The effect of lead on the growth, content of primary metabolites, and antioxidant response of green alga *Acutodesmus obliquus* (Chlorophyceae). Environ Sci Pollut Res 22:19112–19123

Prajapati R, Yadav S, Atri N (2018) Nickel and arsenite-induced differential oxidative stress and antioxidant responses in two *Anabaena* species. J Basic Microbiol 58:1061–1070

Rensing C, Rosen B (2009) Heavy metals cycle (arsenic, mercury, selenium, others). In: Schaeter M (ed) Encyclopedia of Microbiology. Elsevier, Oxford, pp 205–219

Schaedle M, Bassham JA (1977) Chloroplast glutathione reductase. Plant Physiol 59:1011–1012

Singh M, Singh VP, Dubey G, Prasad SM (2015) Exogenous proline application ameliorates toxic effects of arsenate in *Solanum melongena* L. seedlings. Ecotoxicol Environ Saf 117:164–173

Singh JS, Kumar A, Rai AN, Singh DP (2016a) Cyanobacteria: a precious bioresource in agriculture, ecosystem, and environmental sustainability. Front Microbiol 21:529

Singh R, Singh S, Parihar P, Mishra RK, Tripathi DK, Singh VP, Chauhan DK, Prasad SM (2016b) Reactive oxygen species (ROS): beneficial companions of plants' developmental processes. Front Plant Sci 7:1299

Smirnoff N, Wheeler GL (2000) Ascorbic acid in plants: biosynthesis and function. Crit Rev Plant Sci 19:267–290

Srivastava S, Sharma YK (2012) Arsenic induced changes in growth and metabolism of black gram seedlings (*Vigna Mungo* L.) and the role of phosphate as an ameliorating agent. Environ Sci Process 1:431–445

Sure S, Ackland ML, Gaur A, Gupta P, Adholeya A, Kochar M (2016) Probing synechocystis-arsenic interactions through extracellular nanowires. Front Microbiol 7:1134

Upadhyay AK, Mandotra SK, Kumar N, Singh NK, Singh L, Rai UN (2016) Augmentation of arsenic enhances lipid yield and defense responses in alga *Nannochloropsis* sp. Bioresour Technol 221:430–437

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant system in acid rain-treated bean plants. Plant Sci 151: 59–66

Waterhouse AL (2001) Determination of total phenolics in current protocols. In: Wrolstad RE (ed) Food and analytical chemistry. Wiley, USA, pp 1111–1118

Whitton BA (2000) Soils and rice fields. In: Whitton BA, Potts M (Eds.), The ecology of cyanobacteria: their diversity in time and space, Alphen aan den Rijn: Kluwer Academic 233–255

Wu Z, Liu S, Zhao J, Wang F, Du Y, Zou S, Li H, Wen D, Huang Y (2017) Comparative responses to silicon and selenium in relation to antioxidant enzyme system and the glutathione-ascorbate cycle in flowering Chinese cabbage (*Brassica campestris* L. ssp. chinensis var. utilis) under cadmium stress. Environ Exp Bot 133:1–11

Yadav G, Srivastava PK, Parihar P, Tiwari S, Prasad SM (2016) Oxygen toxicity and antioxidative responses in arsenic stressed *Helianthus annuus* L. seedlings against UV-B. J Photochem Photobiol B 165: 58–70

Ye B, Gitler C, Gressel J (1997) A High–sensitivity, single–gel, polyacrylamide gel electrophoresis method for the quantitative determination of glutathione reductases. Anal Biochem 246:159–165

Ye J, Rensing C, Rosen BP, Zhu YG (2012) Arsenic biomethylation by photosynthetic organisms. Trends Plant Sci 17:155–162

Zehr JP (2011) Nitrogen fixation by marine cyanobacteria. Trends Microbiol 19:162–173

Zhao W, Ye Z, Zhao JA (2007) Membrane-associated Mn-superoxide dismutase protects the photosynthetic apparatus and nitrogenase from oxidative damage in the cyanobacterium *Anabaena* sp. PCC7120. Plant Cell Physiol 48:563–572

Zheng Y, Ayotte DJ (2015) At the crossroads: hazard assessment and reduction of health risks from arsenic in private well waters of the northeastern United States and Atlantic Canada. Sci Total Environ 505:1237–1247

Zutshi S, Bano F, Ningthoujam M, Habib K, Fatma T (2014) Metabolic adaptations to arsenic-induced oxidative stress in *Hapalosiphon fontinalis*-339. Int J Innov Res Sci Eng Tech 3:9386–9394