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ARSENIC ACCUMULATION AND ASSOCIATED TOXIC EFFECTS IN *SPIRODELA POLYRHIZA* L.

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Alterations in physiological and biochemical responses under arsenic (As) phytotoxicity were investigated in aquatic model plant, *Spirodela polyrhiza* L. Decrease in dry matter and total chlorophyll was observed in this plant under treatment at increasing concentrations. Similar decrease in protein and carotenoid contents was also recorded compared to the control. Increase in malondialdehyde, an index of lipid peroxidation, membrane damage and ion leakage increased under As treatment as compared to the non-treated plants. Accumulation of As was found to be maximum at higher concentration as compared to the control samples. This study revealed that As imposed stress increased the membrane damage, as is evident by the increased values of electrical conductivity and lipid peroxidation under repeated exposure.

Key words: Accumulation, Lipid peroxidation, Ion leakage, *Spirodela polyrhiza* L.

Arsenic is a major source of food chain contamination in soil and water with respect to plant uptake. It is very essential to know how As is taken up by the roots and metabolized within plants in order to understand and manage the risks posed by soil/water arsenic. Arsenic is present in the environment as both organic and inorganic forms, of which the inorganic form (As III, As V) is more toxic than the organic As. The rate of free radical reactions has been suggested to contribute to the toxicity of metal ions, by affecting membrane function due to sulphydryl reactions and lipid peroxidation (Halliwell and Gutteridge 1993). However, plants possess enzymatic and non-enzymatic antioxidants to protect against the damage caused by these free radicals, keeping the deleterious reactions to a minimum. Antioxidative defense falls into two general classes - (a) low molecular weight antioxidants, which consist of the lipid soluble membrane-associated antioxidants (e.g., α-tocopherol and β-carotene) and the water soluble reductants (e.g., glutathione GSH and ascorbate); and (b) enzymatic antioxidants (e.g., superoxide dismatuse - SOD, catalase CAT, peroxidase - POX, ascorbate peroxidase - APX). Plants living under oxidative stress of heavy metals exhibit adaptive biochemical responses such as production of antioxidant enzymes. In plants, arsenic is accumulated mainly in the root system, to a lesser degree in the aboveground organs, and causes physiological changes and damages (Velikova et al., 2000) and reduction of the crop productivity (Stoeva et al., 2003/4). The role of aquatic plants in the removal of heavy metals is well documented (Zhu *et al.* 2000 and Sinha *et al.* 2002). However, fewer reports are available on the toxic effects induced by As accumulation in aquatic plants like *S. Polyrhiza L.* under repeated exposure.

The objective of this study was to investigate the effects of As in terms of accumulation, electrical conductivity and photosynthesis on an aquatic model plant, *Spirodela Polyrhiza L.*

MATERIAL AND METHODS

Fresh samples of *Spirodela polyrhiza*

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Table 1. Effect of As on dry biomass (g) of *S. polyrhiza* L. at different concentrations and hours of exposure. Data are the mean value ± SE of three independent experiments with triplicates (n=3).*indicates significant difference from the untreated plant at $P < 0.05$.

As (µM)	Exposure (h)		
	12	18	24
0	1.59±0.01	1.57±0.03	1.22±0.02
10	1.40±0.03	1.40±0.01*	1.23±0.04*
50	1.23±0.02	1.32±0.05*	0.24±0.09*
100	1.18±0.04*	1.21±0.03*	0.20±0.12*

Table 2. As accumulation (mg g^{-1} dw) in *S. polyrhiza* L. at different concentrations and hours of exposure. Data are the mean value ± SE of three independent experiments with triplicates **(n=3)**. * indicates significant difference from the untreated plant at $P < 0.05$.

As (µM)	Exposure (h)		
	12	18	24
0	nd	nd	nd
10	28.44±1.18	31.29±1.09*	38.11±1.11*
50	30.12±1.09	36.14±0.11*	41.18±1.21*
100	35.18±1.13*	41.17±1.18*	47.10±1.09*

L. were collected from uncontaminated ponds nearby Assam Central University, Silchar, India. Plants were brought carefully to the laboratory in clean plastic jars. Healthy plants were selected and then cultured in 1:40 (v/v) Hogland's Nutrient solution as suggested by Bassi and Sharma (1993). Plants were cultured in transparent plastic tumblers inside the growth chamber for 7d prior to As treatment and white light with photon flux density of 52 µmol s^{-1} m^{-2} (PAR) was provided with 16h photoperiod. As - treatment in the form of Sodium arsenate was given at concentrations 0, 10, 50 100 µM and plants were harvested after 12, 18 and 24 h. To determine the dry matter, the plants were dried in an oven at 70 °C for 2 days. The dried samples were then weighed to determine the plant dry mass. The total chlorophyll and carotenoid contents were determined spectrophotometrically as described by Lichtenthaler (1987). To determine arsenic, the plants were oven-dried at 70 °C for 2 days. The dried samples were then digested, as described by Humpries (1956), in glass tubes containing 5 ml concentrated HNO_3 and placed in a heat block at 100 °C until the solution became clear. The sample volume was raised to 20 ml by adding deionised water. The concentration of total arsenic in the tissue was measured by an atomic absorption spectrometer (Perkin Elmer-3110, Germany).

The level of lipid peroxidation, expressed as MDA content, was determined as 2-thiobarbituric acid (TBA) reactive metabolites. Plant fresh tissues (0.2 g) were homogenized and extracted in 10 ml of 0.25%

Table 3. Changes in Chlorophyll and Carotenoid contents (mg g^{-1} fw) of *S. polyrhiza* L. at different concentrations and hours of As exposure. Data are the mean value ± SE of three independent experiments with triplicates (n=3). * indicates significant difference from the untreated plant at $P < 0.05$.

As (µM)	Exposure (h)		
	12	18	24
0	2.81±0.18 (0.99 ± 1.01)	2.20±0.14 (0.99 ± 1.01)	2.20±0.18 (0.86 ± 1.04)
10	2.60±0.11 (0.95 ± 0.99)	2.41±0.19 (0.95 ± 0.10) *	2.14±1.12 *(0.88 ± 0.12) *
50	2.50±1.12 (0.95 ± 0.92)	2.11±0.12 (0.78 ± 0.62) *	2.13±1.09 * (0.69 ± 0.35) *
100	1.18±1.31 (0.84 ± 1.02)	1.18±0.18 * (0.63 ± 0.11) *	0.25±0.09 * (0.43 ± 0.31) *

Values in parenthesis are carotenoid contents (mg g^{-1} fw) in *S. polyrhiza* L.

Table 4. Changes in Malondialdehyde (MDA) contents (μ mol g^{-1} fw) in *S. polyrhiza* L. at different concentrations and hours of As exposure. Data are the mean value ± SE of three independent experiments with triplicates **(n=3)**.* indicates significant difference from the untreated plant at $P < 0.05$.

As (μM)	Exposure (h)		
	12	18	24
0	8.92±0.41	8.81±0.11	8.77±0.09
10	9.11±0.93	10.18±0.77	11.91±1.12*
50	9.94±0.07	11.13±0.61*	12.28±0.99*
100	10.44±0.44*	11.81±0.33	12.44±0.18*

TBA made in 10 ml trichloroacetic acid (TCA). Then extract was heated at 95 ◦C for 30 min and then rapidly cooled in ice. After centrifugation at 10,000 *g* for 10 min, the absorbance of the supernatant was measured at 532 nm. Non-specific turbidity was corrected by subtracting the absorbance value taken at 600 nm. The concentration of MDA was calculated using extinction coefficient of 155 mM^{-1} cm^{-1} (Zhang 1992).

The total soluble protein in the supernatant was determined as per the method of Bradford (1976) using BSA as standard. Electrical conductivity was determined as per the method of Dionisio-Sese and Todita (1998) and calculated using the formula-

$EC = EC_1 / EC_2 \times 100$, where EC_1 is the initial conductivity of the sample before autoclaving and EC_2 is the final conductivity of the sample measured after autoclaving the sample.

Statistical analyses: All the observations were done in triplicate, repeated thrice and the data represent mean ± SE. Difference between control and treated plants was analyzed using Student's t-test, taking $P < 0.05$ as significant level (*).

Table 5. Changes in protein contents (μg protein g^{-1} fw) in *S. polyrhiza* L. at different concentrations and hours of As exposure. Data are the mean value ± SE of three independent experiments with triplicates (n=3).* indicates significant difference from the untreated plant at $P < 0.05$.

As (μM)	Exposure (h)		
	12	18	24
0	34.21±1.11	34.28±0.18	32.18±1.12
10	27.17±0.72	33.11±1.14*	22.21±1.08*
50	31.11±0.91	28.14±1.05*	21.18±1.13
100	24.18±0.33*	19.16±0.99	14.28±0.24*

Table 6. Changes in Electrical conductivity (%) in *S. polyrhiza* L. at different concentrations and hours of As exposure. Data are the mean value ± SE of three independent experiments with triplicates **(n=3)**. * indicates significant difference from the untreated plant at $P < 0.05$.

As (μM)	Exposure (h)		
	12	18	24
0	13.2±0.48	13.8±0.12	15.6±1.03
10	16.5±0.22	19.3±0.18*	22.7±1.19
50	20.7±0.31	27.2±0.32	29.4±1.09*
100	25.6±0.09*	31.8±0.44*	34.2±0.18*

RESULTS AND DISCUSSION

A significant growth inhibition measured in terms of dry matter of plant was observed with an increase in As concentrations (Table 1). The inhibition of growth was accompanied by a decrease in chlorophyll and carotenoid contents, which reduced in case of As treated compared to control plants (Table 3). The decrease in dry biomass and chlorophyll content under As in *S. polyrhiza* L. was similar to that of other aquatic and higher plants studied, which may be a metal-specific response on chlorophyll biosynthesis as seen for other heavy metals like Cu, Cd, Cr, Zn etc (Luna *et al.* 1994, Gallego *et al.* 1996, Upadhyay and Panda 2005 and Upadhyay and Panda 2009). On the other hand, the accumulation of As was found to be higher on increasing concentration and exposure against the control plants (Table 2). No As-accumulation could be detected in case of control plants. Though at lower As concentration, accumulation was slow, at higher concentration As showed higher accumulation as seen in higher plants, suggesting that *S. polyrhiza L.* with an efficient bio concentration mechanism (Carginale *et al.* 2004).

Metal-induced membrane deterioration is mostly attributed to increased peroxidation of membrane via increased production of free radicals (Halliwell and Gutteridge 1993). The levels of MDA and the percentage of EC increased significantly with the increased in metal concentrations as compared to the control plants (Table 4 and 6). Similarly, metals like Cu and Ni are known to induce lipid peroxidation in plants (Rao and Sresty 2000 and Upadhyay and Panda 2009). In the present experiments, increase in MDA content in *Spirodela* plants, indicated lipid peroxidation via free radical generation caused possibly by enhanced production of H_2O_2 and O^-_2 . This result is in accordance with the findings in *Ceratophyllum dermersum* L. and *Pistia stratiotes* L. (Devi and Prasad 1998 and Upadhyay and Panda 2009). However, the percentage of EC of the plant increased with increasing dose as well as duration of exposure against the control values, which indicated the increase in EC. This may be due to ion leakage caused by the membrane damage resulting from As exposure. Protein content in an organism is an important indicator of reversible and irreversible changes in metabolism, known to respond to a wide variety of stressors such as natural and xenobiotic (Hou *et al.* 2007). A concentration and time dependent increased with increasing higher concentration of total protein, was observed in Table 5. The protein content gradually increases as compared to control. The inability of *S. polyrhiza L.* to synthesize protein after As exposure might have caused by acute oxidative stress induced by elevated concentration in plant cells, as also reported in other aquatic plants like *Lemna minor* L. and *Pistia stratiotes* L. under cuexposure (Hou *et al.* 2007 and Upadhyay and Panda 2009). It revealed that As imposed oxidative stress increased the membrane damage, as evident by the increased levels of MDA and electrical conductivity, but decreased chlorophyll, carotenoid and protein contents may help the plants to live on with stress under exposure.

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