➔DOI : 10.15740/HAS/AJBS/11.1/32-36
e ISSN-0976-8343 | ■ Visit us : *www.researchjournal.co.in* ■ – AFBSAH-2016 ASIAN JOURNAL OF BIO SCIENCE
Volume **11** | Issue 1 | April, 2016 | 32-36

RESEARCH **P**APER

Phytoremidiation of *Ceratophyllum demersum* L.on arsenate and cadmium exposure

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Article Info : Received : 10.12.2015; Accepted : 06.02.2016

In the present study plants of *Ceratophyllum demersum* L. was collected and grown for six months in large hydrophobic tubes. The effect of zinc ion concentration was studied at different concentration on plant; for 7 days in 10 per cent Hoagland media. After day 1 no significant effect was observed on plant for all concentration of zinc. After day 2, 3 and 4 change in colour from green to yellow was observed with different colour intensity. It was observed that after day 5, 6 and 7 the leaves of *Ceratophyllum demersum* L. become black in colour, the intensity of blacking in colour was increased as concentration of zinc ion increased.The plant showed maximum accumulation of cadmium after 7 day at 20µM concentration.The maximum level of thiol compound was observed at 10µM after 3 days. The maximum level of cysteine synthetase was observed at 10µM after 3 days. The maximum level of glutathione-S-transferase was observed at 10µM after 4 days. The maximum level of glutathione reductase was observed at 10µM after 4 days. The plant showed maximum accumulation of arsenic after 7 day at 20µM concentration. The maximum level of cysteine concentration was observed at 15µM after 4 days. The maximum reduced glutathione concentration was observed at 10µM and 20µM, respectively.

Key words : *C. demersum*, Arsenat, Thiol metabolism, Cysteine synthase, Glutathione S-transferase, Glutathione reductase

How to cite this paper : Gautam, Aparna, Singh, Poonam and Lall, Rohit (2016). Phytoremidiation of *Ceratophyllum demersum* L. on arsenate and cadmium exposure. *Asian J. Bio. Sci.*, **11** (1) : 32-36 [Special Issue of AFBSAH-2016].

INTRODUCTION

Water contamination with heavy metals is a very important problem in the present world. Use of such aquatic food stuff enriched with toxic metals may cause serious health hazards through food-chain enhacment (Khan *et al.,* 2000).

It is a well-known fact that aquatic plants accumulate metals that they take from the environment and concentrate on the trophic chains with accumulative result (Outridge and Noller, 1991). Accumulation and distribution of metal have important consequences in the capacity and removal of metal rate (Ellis *et al.,* 1994).

Toxicity of Cd may result from its binding to sulfydryl groups of proteins leading to inhibition of activity or structural disruption, cellular redox control disturbance (Schutzendubel and Polle, 2002), and/or generate the production of reactive oxygen species. From various detoxification pathways activated in plants under the stress of heavy metal, raise the synthesis of sulfur containing defense compounds namely, cysteine, glutathione (GSH) and phytochelatins (PCs) is considered to be of prime importance for the tolerance and survival of plants (Rausch and Wachter, 2005 and Grill *et al.,* 2006). The tolerance to heavy metal toxicity has been found to be correlated to the level of thiols (Cobbett, 2000), however, this induces requirement for the reduced sulfur. This expansion in demand is reflected by both increased

activities of sulfate assimilation enzymes and an elevated expression of genes encoding these proteins. They are found in plants which are exposed to Cd stress. Cysteine is synthesized by the enzyme cysteine synthase (CS) at the final step of sulfate assimilation pathway and high cysteine biosynthesis rate has been demonstrated to increase the synthesis of downstream peptides GSH and PCs under stress conditions (Dominguez-Solis *et al.,* 2001).

In detoxification mechanisms the oxidation of GSH to form oxidized glutathione(GSSG) takes place. Therefore, the role of glutathione reductase (GR), which reduces GSSG back to GSH, becomes essential for the maintenance of the redox state of the cells under metal stressed conditions (Mishra *et al.,* 2006). Arsenic toxicity can be found both in water and food chain contamination (Mondal *et al.,* 2006 and Tripathi *et al.,* 2007). Plant of *C. demersum* L. (family Ceratophyllaceae) is selected for the experiment which is a rootless aquatic plant. Present topic deals with the comparative study of phytoremidiation of *Ceratophyllum demersum* on arsenate and cadmium exposure.

RESEARCH METHODOLOGY

Plant materials and treatment conditions :

Plants of *C. demersum* were collected from the local shop and were grown in large hydroponic tubes and used to determine various parameters.

Quantification of arsenic :

Arsenic concentrations were determined on UV – Visible double beam spectrophotometer at 230nm (Abedin *et al.,* 2002).

Quantification of cadmium :

Cadmium concentration was determined on a UV-Visible double beam spectrophotometer at 229 nm by following standard protocol.

Estimation of thiol compound on cadmium exposure:

The reaction mixture containing 0.5ml of aq. cysteine hydrochloride solution (0.05-0.5µ mole), 0.5ml of acetic acid and 0.5ml of acid ninhydrin reagent 1 or 2, was mixed thoroughly. The reaction may be performed with a sample volume of 1.0ml or 1.5ml. The tubes were covered with aluminum caps or glass marbles and heated in a boiling-water bath for 10min. They were then rapidly cooled in tap water; the contents of the tube were diluted to 5 or 10ml with 95 per cent ethanol and mixed. A reagent blank without cysteine was prepared under the same conditions. The spectral measurements of the reaction products were made against the reagent blank at 350nm using double beam spectrophotometer.

Estimation of thiol compounds in arsenate treated plant :

Effect of various concentration of arsenate on non-protein thiol compound was studied such as reduced glutathione and oxidized glutathione. The level of reduced glutathione was studied spectrophotometrically at 340nm using Bradford assay using o-phthaldialdehyde.

Assay of cysteine synthetase on cadmium exposure:

The amount of cysteine synthesized was determined by the Bradford assay using BSA as standard.

Estimation of cysteine in arsenic treated plant :

The reaction mixture was measured spectrophotometerically at 350nm using double beam spectrophotometer using reagent ninhydrin reagent 1 or 2 AFSA (Habeeb,1966)

Assay of glutathione-S-transferase from plant treated with cadmium :

The protein content in the supernatants was measured according to Bradford assay.

Assay of glutathione reductase from cadmium treated plant :

The amount of glutathione reductase synthesized was determined spectrophotometrically at 412 nm by the Bradford assay using BSA as standard.

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Effect of cadmium on *Ceratophyllum demersum* :

Accumulation of Cd by *C. demersum* plants was found to be correlated to both concentration and duration of the treatment. The plant showed maximum accumulation of cadmium after 7 day at 20µM concentration (Table 1).

Accumulation of arsenic and its effect on *Ceratophyllum demersum* :

Accumulation of arsenic was found to depend on both concentration and duration of exposure and showed a linear increase. The plant showed maximum accumulation of arsenic after 7 day at 20µM concentration (Table 2).

Estimation of thiol compound from plant treated with cadmium :

The level of thiol compounds increased significantly at all the cadmium exposure concentrations. The maximum level of thiol compound was observed at 10µM after 3 days (Table 3).

Assay of thiol compound (reduced glutathione) from plants treated with arsenic :

The level of reduced glutathione compounds increased significantly at all the arsenate exposure concentrations.

Assay of thiol compound (oxidized glutathione) plant treated with arsenate :

The level of oxidized glutathione compounds

Table 1: Effect of cadmium on *Ceratophyllum demersum* after 1,2,3,4,5,6 and 7 days

Sr.No.	Conc. of cadmium	Effect of cadmium						
		1days	2 days	3 days	4 days	5 days	6 days	7 days
1.	0.00 µM	0.00µg	0.00 µg	0.00 µg	0.00 µg	0.00 µg	0.00 µg	0.00 µg
2.	1µM	131 µg	162 µg	168 µg	174 µg	189 µg	209 µg	234 µg
3.	5 µM	136 µg	164 µg	173 µg	182 µg	194 µg	224 µg	252 µg
4.	10 µM	157 µg	188 µg	194 µg	202 µg	199 µg	243 µg	297 µg
5.	15 µM	173 µg	196 µg	217 µg	247 µg	214 µg	288 µg	320 µg
6.	20 µM	262 µg	281 µg	290 µg	310 µg	369 µg	376 µg	417 µg

Table 2: Estimation of arsenate ion concentration in *Ceratophyllum demersum* after 1,2 3, 4, 5, 6 and 7 days

Sr. No.	Conc. of arsenic	Estimation of arsenic after						
		1days	2 days	3 days	4 days	5 days	6 days	7 days
1.	0.00 µM	0.00µg	0.00µg	0.00µg	0.00µg	0.00µg	0.00µg	0.00µg
2.	1µM	157 µg	168 µg	176 µg	179 µg	198 µg	213 µg	236 µg
3.	5 µM	162 µg	173 µg	181 µg	194 µg	209 µg	247 µg	276 µg
4.	10 µM	188 µg	194 µg	207 µg	222 µg	225 µg	262 µg	289 µg
5.	15 µM	194 µg	210 µg	215 µg	274 µg	287 µg	296 µg	306 µg
6.	20 µM	202 µg	245 µg	263 µg	299 µg	313 µg	344 µg	352 µg

Table 3: Estimation of thiol compound on *Ceratophyllum demersum* after day 1, 2, 3, 4, 5, 6 and 7 days

Sr.No.	Conc. of cadmium	Thiol estimation						
		1days	2 days	3 days	4 days	5 days	6 days	7 days
1.	0.00 µM	1.2	1.3	1.4	1.5	1.2	1.2	1.25
2.	1µM	1.6	2.3	2.9	3.2	2.4	1.9	1.8
3.	5 µM	2.91	2.52	3.3	3.01	2.39	2.34	2.33
4.	10 µM	3.0	3.1	3.4	2.9	2.6	2.57	2.43
5.	15 µM	2.7	2.6	2.65	2.46	2.41	2.33	2.29
6.	20 µM	2.2	1.9	1.9	1.6	1.2	1.25	1.27

Table 4: Estimation of oxidized glutathione in *Ceratophyllum demersum* after 1, 2, 3, 4, 5, 6 and 7 day

Sr. No.	Conc. of arsenic	Concentration of GSSH						
		1days	2 days	3 days	4 days	5 days	6 days	7 days
1.	1µM	0.20	0.26	0.27	0.29	0.28	0.26	0.23
2.	5 µM	0.23	0.30	0.31	0.32	0.29	0.27	0.25
3.	10 µM	0.31	0.34	0.34	0.42	0.39	0.31	0.27
4.	15 µM	0.32	0.36	0.39	0.52	0.48	0.32	0.33
5.	20 µM	0.35	0.40	0.45	0.55	0.54	0.5	0.36

increased significantly at all the arsenate exposure concentrations. The maximum level of concentration was observed on 4th day at 20µM (Table 4).

Assay of cysteine synthetase from plant treated with cadmium :

The level of cysteine synthetase compounds increased significantly at all the cadmium exposure concentrations. The maximum level of cysteine synthetase was observed at 10µM after 3 days (Table 5).

Assay of cysteine from plant treated with arsenic :

The level of cysteine increased significantly at all the arsenate exposure concentrations (Table 6).

Assay of glutathione-S-transferase from plant treated with cadmium :

The level of glutathione reductase compounds increased significantly at all the cadmium exposure

Table 5: Estimation of cysteine synthetase on *Ceratophyllum demersum* after day 1, 2, 3, 4, 5, 6 and 7 days

Sr. No.	Conc. of cadmium	Concentration of CS						
		1days	2 days	3 days	4 days	5 days	6 days	7 days
1.	0.00 µM	0.85	0.97	1.40	0.36	0.36	0.17	0.17
2.	1µM	1.03	1.34	1.58	0.85	0.91	0.66	0.54
3.	5 µM	1.03	1.58	1.76	1.21	1.09	0.97	0.42
4.	10 µM	0.60	0.54	2.01	0.97	0.72	0.48	0.36
5.	15 µM	0.36	0.66	1.21	0.17	0.17	0.66	0.17
6.	20 µM	0.30	0.36	0.97	0.05	0.30	0.66	0.05

Table 6: Estimation of cysteine concentration in *Ceratophyllum demersum* after days 1 , 2, 3, 4, 5, 6 and 7 days

Sr. No.	Conc. of arsenic	Cysteine estimation after						
		1days	2 days	3 days	4 days	5 days	6 days	7 days
1.	0.00 µM	2.65	3.13	3.315	4.55	5.06	4.85	3.52
2.	1µM	4.28	5.84	6.05	6.79	5.80	6.18	3.90
3.	5 µM	5.54	5.93	6.18	7.96	7.13	6.94	2.63
4.	10 µM	8.84	9.25	9.41	9.54	8.84	7.74	2.99
5.	15 µM	12.12	12.16	12.31	12.67	10.36	9.22	3.20
6.	20 µM	6.02	6.12	6.18	8.08	4.47	3.90	2.76

Table 7: Estimation of glutathione-S-transferase on *Ceratophyllum demersum* after 1, 2, 3, 4, 5, 6 and 7 days

Sr. No.	Conc. of cadmium	Concentration of GST						
		1days	2 days	3 days	4 days	5 days	6 days	7 days
1.	0.00 µM	0.82	1.10	0.95	1.20	0.69	0.50	0.22
2.	1µM	1.13	1.26	1.20	1.26	0.79	0.60	0.10
3.	5 µM	0.85	1.48	1.07	1.20	1.07	0.63	0.41
4.	10 µM	1.20	1.01	0.95	1.73	0.85	0.66	0.73
5.	15 µM	0.47	0.88	1.07	0.95	0.47	0.41	0.35
6.	20 µM	0.63	0.60	0.79	0.69	0.47	0.35	0.32

Table 8: Estimation of glutathione reductase on *Ceratophyllum demersum* after 1, 2, 3, 4, 5, 6 and 7 days

Sr. No.	Conc. of cadmium	Concentration of GR						
		1days	2 days	3 days	4 days	5 days	6 days	7 days
1.	0.00 µM	0.66	1.04	1.48	1.61	0.54	0.32	0.35
2.	1µM	1.13	1.23	1.57	1.89	0.60	0.35	0.32
3.	5 µM	0.91	1.39	1.67	2.17	0.88	0.57	0.60
4.	10 µM	0.79	0.95	1.80	2.30	1.10	0.63	0.50
5.	15 µM	0.69	0.82	1.39	1.92	0.95	0.54	0.44
6.	20 µM	0.60	0.54	1.26	1.51	0.88	0.47	0.38

concentrations. The maximum level of Glutathione-S-transferase was observed at 10µM after 4 days (Table 7).

Assay of glutathione reductase from cadmium treated plant :

The level of glutathione reductase compounds increased significantly at all the cadmium exposure concentrations. The maximum level of glutathione reductase was observed at 10µM after 4 days (Table 8).

In the present study, the level of thiols and activity of related enzymes were investigated in *Ceratophyllum demersum* plants to analyze their role in combating the stress caused upon exposure to cadmium (Cd; 0–10µM) for duration up to 7 days. Plants showed the maximum accumulation after 7 days. Significant increases in the level of total non-protein thiols (NPSH) as well as upstream metabolites of the PC biosynthetic pathway, cysteine and glutathione (GSH) were observed. In addition, significant increases in the activities of cysteinesynthase (CS), glutathione-S-transferase (GST), glutathione reductase (GR), were noticed in response to Cd.

Hence, it is concluded that *Ceratophyllum demersum* L. is known to be a potential accumulator of arsenic (As), in the present study; it was analyzed that biochemical responses of *Ceratophyllum* plants to arsenate exposure to explore the underlying mechanisms of As detoxification. Exposure of plants to higher concentrations (250µMAsV) and/or for longer durations (7 days) resulted in a significant increase in the level of As and an inverse relationship between As accumulation and various detoxification strategies was observed that lead to enhanced oxidative stress and hampered growth.

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