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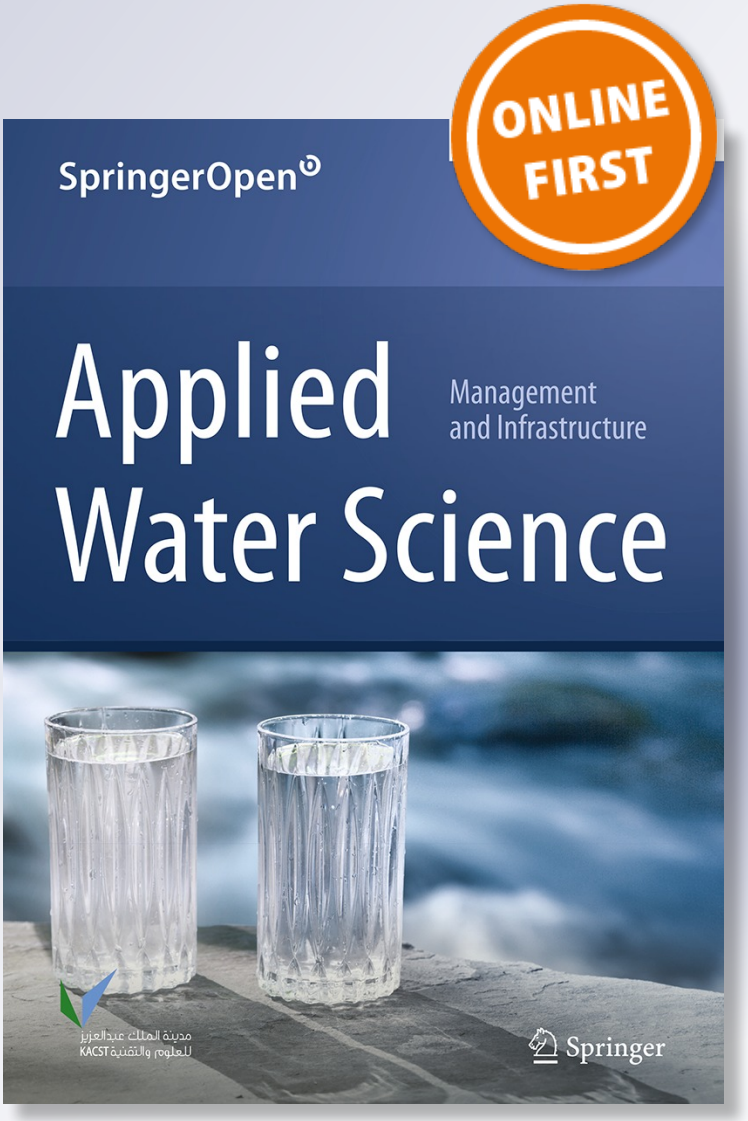

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ORIGINAL ARTICLE

Performance of aquatic plant species for phytoremediation of arsenic-contaminated water

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Abstract This study investigates the effectiveness of aquatic macrophyte and microphyte for phytoremediation of water bodies contaminated with high arsenic concentration. Water hyacinth (*Eichhornia crassipes)* and two algae (*Chlorodesmis* sp. and *Cladophora* sp.) found near arsenic-enriched water bodies were used to determine their tolerance toward arsenic and their effectiveness to uptake arsenic thereby reducing organic pollution in arsenic-enriched wastewater of different concentrations. Parameters like pH, chemical oxygen demand (COD), and arsenic concentration were monitored. The pH of wastewater during the course of phytoremediation remained constant in the range of 7.3–8.4, whereas COD reduced by 50–65 % in a period of 15 days. *Cladophora* sp. was found to survive up to an arsenic concentration of 6 mg/L, whereas water hyacinth and *Chlorodesmis* sp. could survive up to arsenic concentrations of 2 and 4 mg/L, respectively. It was also found that during a retention period of 10 days under ambient temperature conditions, *Cladophora* sp. could bring down arsenic concentration from 6 to <0.1 mg/L, *Chlorodesmis* sp. was able to reduce arsenic by 40−50 %; whereas, water hyacinth could reduce arsenic by only 20 %. *Cladophora* sp. is thus suitable for co-treatment of sewage and arsenic-enriched brine in an algal pond having a retention time of 10 days. The identified plant species provides a simple and cost-effective method for application in rural areas affected with arsenic problem. The treated water can be used for irrigation.

Keywords Phytoremediation · Water treatment for arsenic removal · Water hyacinth · Algae

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Introduction

The occurrence of arsenic has been marked in groundwater supplies in several regions in more than 70 countries and over 150 million people are estimated to be exposed to arsenic, predominantly belonging to rural areas (Shankar et al. 2014). The treatment technology for arsenic removal includes electro dialysis, ion exchange, ultrafiltration, etc., which produces arsenic-enriched water rejects. The outcome of these technologies is potable water along with arsenic-rich wastewater. This waste is generally stockpiled and thrown or disposed in nearby surroundings that could lead to leaching of arsenic back into the soil and water system making groundwater more susceptible to arsenic concentrations. Management of arsenic-rich waste from these systems is a major environmental concern (Magalhaes 2002). New sustainable techniques need to be identified to address removal of arsenic residues in the water environment.

Phytoremediation of toxins from aquatic environment is gaining popularity as a low-cost environment-friendly

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technology for decentralized wastewater treatment. This study aims to identify suitable aquatic species that can uptake arsenic out of water and bio-accumulate it in its tissues or membranes. This study further evaluates the efficiency of arsenic removal by the identified species and retention time required by them for effective removal under the condition of arsenic-enriched wastewater mixed with domestic sewage. Specific growth rate of aquatic species and chemical oxygen demand (COD) removal rate is also simultaneously evaluated. Phytoremediation is done using three principle methods as in situ, in vivo, and in vitro (Susarla et al. 2002). Community-level wastewater treatments, where the volume of wastewater is high, principally rely on in situ methods as it is least expensive and easy to maintain (Erakhrumen and Agbontalor 2007). Phytoremediation techniques use any one of the six mechanisms such as phytoaccumulation/phytoextraction, phytotransformation, phytostabilization, phytovolatilization, phytostimulation, and rhizofiltration (Rahman and Hasegawa 2011; Erakhrumen and Agbontalor 2007). Among these, in situ-type phytoextraction mechanism is mostly preferred for heavy metals as in vivo and in vitro techniques are more expensive (Susarla et al. 2002). In vivo involves transferring of contaminant from contaminated site to a treatment plot area where plants are added for remediation applications. When above two methods fail in vitro is preferred in which extracts (primarily in the form of enzymes) from live plants are added to the contaminated sites (Susarla et al. 2002). This method thus involves precision of advanced scientific technology making it an expensive treatment method.

The success of the chosen method depends on proper selection of plant species that has: (1) high specific growth rate in the contaminated environment, (2) large specific surface area of the portion in contact with water, and (3) high translocation potential (Nazir et al. 2011). Alternatively, factors like bio-concentration factor (BCF) and translocation factor (TF) also relate to the plants' sensitivity for phytoremediation. Plants with more than one BCF and high root-to-shoot metal translocation, as displayed by brake fern (*Pteris vittata*), are ideal for phytoremediation and also relate to the plant to act as a hyper-accumulator (Pandey 2012; Hadi et al. 2014). For instance, plant species like duckweed (*Lemna gibba*), water spinach (*Ipomonea aquatica*), and fern (*Azolla pinnata*) have been reported to phytoremediate metals like boron, chromium, and manganese, respectively (Marin and Oron 2007; Bharti and Banerjee 2012; Chen et al. 2010).

Aquatic macrophytes like water hyacinth have been extensively used for phytoremediation of water contaminated with dyes (Khaiary 2007) and metals like cadmium, arsenic, lead, and chromium (Agunbiade et al. 2009). Hasan et al. (2007) reported effectiveness of water hyacinth for sorption of zinc (II) and cadmium (II) from aqueous solutions up to a concentration of 6 and 2.5 mg/L, respectively. On the other hand, aquatic macrophytes like duckweed not only phytoremediate but also transform pollutants. For example, Lemnaceae family species remove dyes like Acid blue (azo dye AB92) and transform it into different intermediate compounds (Khataee et al. 2012). Another duckweed species *Spirodela polyrhiza* is effective for arsenic sorption via phosphate uptake pathway (Rahman et al. 2007). Other aquatic plants for phytoremediation are *Azolla* (*w*ater *f*ern) and *Hydrilla verticillata* for Fly ash and uranium, respectively (Pandey 2012; Srivastava et al. 2010). Recently, aquatic plant *Micranthemum umbrosum* has also been witnessed for arsenic and cadmium removal by phytofilteration method (Islam et al. 2015). Also *Oenothera picensis* plant has been studied for phytoextraction of copper (Gonzalez et al. 2014).

Algae are also effective for phytoremediation of metals, for example, charaphytes like *Chara aculeolata* and *Nitella opaca* can be used to remove cadmium, lead, and zinc (Sooksawat et al. 2013). Among the selected species, *Chara aculeolata* showed a better performance by >95 % metal reduction as compared to *Nitella opaca*. A marine brown algae *Cystoseira indica* after chemical treatment is effective against chromium. About 20.9–27.9 mg uptake of chromium by a gram of algae biomass was observed (Basha et al. 2008). Studies have revealed the mechanism of metal uptake by algae species like blue-green algae *Spirulina* sp. is chemisorption (chemical adsorption) of metals like chromium and copper other than physical adsorption (Chojnacka and Wojciechowski 2007). Species like *Ranunculus trichophyllus*, *Ranunculus peltatus*, *Lemna minor*, *Azolla caroliniana* have the potential to serve as arsenic indicators (Favas et al. 2012).

Among macrophytes, water hyacinth has more uptake capacity then duckweed (Alvarado et al. 2008). Both species were able to sustain for 14 days after which desorption starts. *Ulothrix cylindricum* (green algae), showed a biosorption capacity of 67.2 mg/g which proved that this alga can be used as an effectual and cost-effective method of biosorption of arsenic (III) from solutions (Tuzen et al. 2009). Promising results for use of filamentous alga species for arsenic biosorption by green algae *Cladophora* sp. have also been reported with nearly 0.36 % by weight arsenic found at active absorption sites (Jasrotia et al. 2014). The study also confirmed the attachment of arsenic at active absorption sites with post-transformation of arsenic to arsenosugars. Arsenic in algae is generally found to be present as organic arsenic (Jasrotia et al. 2014; Diaz et al. 2012).

Most of the studies on algae and aquatic plants have used microwave-assisted dried powdered form of these species (Pell et al. 2013). No study for living algae and water hyacinth species has been done for arsenic removal

from water. Therefore, investigations are required to identify most suitable species by their tolerance for arsenic, their duration of sustenance, arsenic uptake capacity, and simultaneously organic pollution reduction capacity. The results would thus help for upgrading waste stabilization pond for co-treatment of arsenic wastewater in rural locations. The effectiveness of water hyacinth (*Eichhornia crassipes*) and two locally available algae, *Chlorodesmis* sp. and *Cladophora* sp., was studied for phytoremediation of arsenic under similar conditions so as to make a proper choice among these for application for mass-scale remediation in a cost-effective way. Studies have been conducted with respect to their survival under different concentrations of arsenic in water, growth rate, organic pollution reduction, and the arsenic removal efficiency.

Materials and methods

Collection of plant species, algal biomass, and their acclimatization

Sexually reproducing water hyacinth plants were randomly collected from Yamuna river bank, New Delhi (28°5′N and 77°2′E) near Okhla barrage where water remains stagnated for most part of the year. The green plants found floating as mats on the water surface were collected with care so as to avoid breakage and damage to fibrous roots. This is important as maximum metal absorption takes place in the root system. They were stored with locally available water in polythene bags which were sealed air tight at the site and brought to the laboratory within 2 h. They were cleaned with distilled water to remove the attached dirt and soil and were further maintained in tap water with added nutrients (potassium and nitrate salts) till further experimentation. Dead parts of the plant visible in the form of yellow leaves and drooping stems were removed. Finally, plants of similar size, shape, and height (roots 2–6 cm; aerial parts 6–12 cm; weight 80 ± 5 g) were selected for further experiments (Soltan and Rashed 2003).

Two different free floating varieties of algae, that is, slimy and non-slimy were collected from the same location. They were brought to the laboratory and washed with distilled water to remove dust and impurities. The samples were maintained in similar conditions as for water hyacinth till further experimentation. Further microscopic investigations (Alpert et al. 1984) were done to identify the species.

Preparation of experiment solutions

Experimental solutions enriched with arsenic were prepared by adding stock solutions of $NaAsO_2$ and $Na_2HAsO_4{\cdot}7H_2O$ (APHA 1998) in local groundwater so as to achieve three levels of final arsenic concentrations, viz., 2, 4, and 6 mg/L. Each solution was supplemented with nutrients such as KNO_3 (0.5 gm/L), KH_2PO_4 (0.2 gm/L), $MgSO_4{\cdot}7H_2O$ (0.2 gm/L), and $CaCl_2{\cdot}2H_2O$ (0.1 gm/L) and sewage. The ratio of groundwater and sewage was kept as 1:1. The solution for control experiments had 0 mg/L of arsenic concentration. Evaporation losses during the course of experiment were compensated by adding distilled water every alternate day.

Experimental setup

Experiments were performed in plastic tubs of 10.8 L capacity. To test the survival of different species in arsenic-enriched water, the tubs were filled with 4 L of experimental solutions. All the three species were exposed to arsenic concentration of 0, 2, 4, 6, mg/L for 15 days under ambient sunlight. All experimental solutions were made in triplicate for each of the selected aquatic species, and thus a total of 12 tubs were used simultaneously for a single run of the experiment. Total three runs were done to confirm reproducibility of results.

About 80 ± 5 g (wet wt.) of water hyacinth and 80 ± 15 g (wet wt.) of algae were added in respective tubs at start of the experiment. Growth of water hyacinth and algae were monitored visually and quantitatively every alternate day. Visual assessment of water hyacinth was done by observing changes in the general appearance of the plant such as number of new pods, color of leaves, stolon, and wilting. Quantitative assessment involved measuring the change in wet weight of the plant. Plants with green leaves (G) were considered as healthy plants (H), whereas plants with yellow leaves (YL) were considered as unhealthy plants (UH). Similarly, plants with non-healthy leaf appearance which showed wilting were termed as dead plant (DP). Further, algae were inspected for change in color and density. Plants were marked as unhealthy (UH) when there were visible patches of green and brown and they were termed as dead algae (DA) when color appeared brown throughout. Density was measured by estimation for total chlorophyll (*Chl A*) concentrations (mg/g) using spectrophotometer at wavelengths of 664 and 647 nm, respectively (Lim et al. 2010).

Species showing survival were monitored for their growth rate and arsenic uptake rate. The experiments were performed in a similar procedure as used for testing survival rates. The specific growth rate of algae was measured using Eq. (A.1) and water hyacinth was measured for its change in weight per unit time. Arsenic removal rate was measured by noting the residual arsenic concentration of the experimental solution every alternate day. Three runs of the experiment were performed one after the other to test the reproducibility.

$$\text{S, Day}^{-1} = (\ln N_2 - \ln N_1)/(t_2 - t_1), \quad (1)$$

where S, N_1, and N_2 represent the *Chl A* concentrations at times t_1 (initial day) and t_2 (final day), respectively. Eq. (1).

Water quality analysis

Every alternate day, 50 mL of water samples were taken from each tub for water quality analysis. Arsenic was analyzed using atomic absorption spectrophotometer and pH and COD were analyzed as per standard methods (APHA 1998). Quality control of analysis was carried out by using sample replicates and blanks.

Statistical analysis

Descriptive statistics were made using Excel (Microsoft Inc.) software package. Analysis of variance (ANOVA) was performed to assess whether or not the treatments influenced arsenic absorption and to register any difference in fresh and dry mass gain by the species. All analytical results were performed as the average of the replicates.

Results and discussion

Identified algae were *Chlorodesmis* sp. and *Cladophora* sp. based on their round reticular chloroplast structure. Water hyacinth and both algal species were healthy and green in color at the start of the experiment. Table 1 shows the survival of different species in arsenic-enriched water.

At the end of the experiment (Day 15), the water hyacinth survived only in arsenic concentration of 2 mg/L; although wilting appeared from Day 14 onward both under control conditions and in arsenic-enriched water. It is noteworthy that studies by Alvarado et al. (2008) also showed water hyacinth plant tissue death after 14 days where they conducted experiments to assess the bioremediation of arsenic from water containing 0.15 mg/L arsenic. Although the present findings showed that the water hyacinth survived up to Day 15 only in arsenic concentrations of 2 mg/L and death occurred earlier with increasing arsenic concentrations, these can be compared with the results from Ingole and Bhole (2003) who concluded that by using water hyacinth, arsenic could be effectively removed from wastewater when its concentration was less than 10 mg/L. However, growth of the plant during its survival period is found to be negligible (Fig. 1a). On the other hand, *Chlorodesmis* sp. survived in arsenic concentration of up to 4 mg/L and *Cladophora* sp. survived in arsenic concentration of up to 6 mg/L. Specific growth rate of *Cladophora* sp. was found to be higher than *Chlorodesmis* sp. in all conditions (S, $\text{day}^{-1} = 0.10$) at an arsenic concentration of 2 mg/L (Fig. 1b). One-way ANOVA analysis revealed a significant difference for arsenic uptake in all the three aquatic species at 2 mg/L arsenic concentration ($p < 0.01$). Also, for arsenic concentration of 4 mg/L, there is a significant difference for arsenic uptake by both the algal species ($p = 0.006$).

The pH of water in all samples throughout the experimentation was found in the range of 7.3–8.4 and no distinct pattern of fluctuation was observed. Changes in the concentration of COD and of arsenic concentrations with water hyacinth, *Chlorodesmis* sp., and *Cladophora* sp. are shown in Figs. 2, 3, 4, respectively.

About 50 % removal efficiency for COD was observed in use of water hyacinth. The plant was able to survive in arsenic concentration of up to 2 mg/L, whereas the uptake of arsenic by the water hyacinth was only about 20 % and desorption of arsenic into the water was observed from the 9th day onward. For *Chlorodesmis* sp., nearly 50−55 % COD removal was found. The, arsenic uptake was nearly 40−50 % with desorption observed from 11th day onward.

On the other hand, removal efficiency by COD for *Cladophora* sp. was slightly better than other two species and found to be in range of 55–60 %. COD removal rate was high in the initial 10 days and after that no significant removal of COD was observed. This pattern of COD removal coincided with the sharp decline in specific growth rate observed from Day 10th onward. Up to 99 % arsenic uptake was found for *Cladophora* sp. and desorption was observed from 14th day. Similar results for arsenic uptake by *Cladophora* sp. were also obtained by Pell et al. (2013). The strong tolerance or retention time of algae in arsenic-rich water is due to its strong defence mechanism against possible oxidative damages inside the cell structure (Pinto et al. 2003). This can be explained on the basis of the mechanism put forth by Arunaumara and Xuecheng (2008). According to them, the metal uptake by plant species depends on cell surface interactions and intercellular accumulation. Heavy metals enter the cells by active transport or by endocytosis where metal binding to sulphydryl proteins or disruption of protein structure and displacement of necessary elements takes place. Intercellular binding of arsenic to algae takes place by the biomethylation pathway. This involves enzymes like reductases and methyltransferases (such as AS3MT) which are responsible for arsenic biomethylation. This enzyme catalyzes the transfer of the methyl group from *S*-adenosylmethionine (SAM) to trivalent arsenic (Shen et al. 2013).

Table 1 Survival of different species in arsenic-enriched water

Duration									
Arsenic conc. (mg/L)	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 15
Water hyacinth									
0	H, G	H, G	H, G	H, G	H, G	H, G	H, G	H, G + YL	UH
	(81.7 g)	(81.97 g)	(79.88 g)	(70.93 g)	(62.14 g)	(48.56 g)	(40.4 g)	(28.3 g)	(25.3 g)
2	H, G	H, G	H, G	H, G	H, G	H, G + YL	H, G + YL	UH	UH
	(82.2 g)	(81.1 g)	(75.4 g)	(71.82 g)	(69.05 g)	(59.65 g)	(41.26 g)	(20.5 g)	(18.1)
4	H, G	H, G	H, G	UH	UH	UH	UH	DP	DP
	(80.3 g)	(78.2 g)	(76.5 g)	(52 g)	(39.3 g)	(28.9 g)	(23.4 g)	–	–
6	H, G	H, G	UH	UH	DP	DP	DP	DP	DP
	(78.4 g)	(63 g)	(47 g)	(26.4 g)	–	–	–	–	–
Chlorodesmis sp.									
0	H, G	H, G	H, G	H, G	H, G	H, G	H, G	H, G	H, G
	(0.02)	(0.04)	(0.06)	(0.06)	(0.08)	(0.11)	(0.16)	(0.18)	(0.13)
2	H, G	H, G	H, G	H, G	H, G	H, G	H, G	UH	DA
	(0.02)	(0.03)	(0.09)	(0.11)	(0.11)	(0.11)	(0.09)	(−0.04)	–
4	H, G	H, G	H, G	H, G	H, G	H, G	H, G	UH	DA
	(0.02)	(0.04)	(0.05)	(0.09)	(0.05)	(0.04)	(0.01)	–	–
6	H, G	H, G	H, G	UH	DA	DA	DA	DA	DA
	(0.02)	(0.05)	(0.07)	(−0.05)	–	–	–	–	–
Cladophora sp.									
0	H, G	H, G	H, G	H, G	H, G	H, G	H, G	H, G	H, G
	(0.03)	(0.05)	(0.06)	(0.07)	(0.09)	(0.11)	(0.16)	(0.19)	(0.18)
2	H, G	H, G	H, G	H, G	H, G	H, G	H, G	UH	UH
	(0.03)	(0.05)	(0.06)	(0.07)	(0.08)	(0.12)	(0.09)	(−0.04)	(−0.02)
4	H, G	H, G	H, G	H, G	H, G	H, G	H, G	UH	UH
	(0.03)	(0.05)	(0.06)	(0.06)	(0.12)	(0.09)	(0.09)	(−0.04)	(−0.02)
6	H, G	H, G	H, G	H, G	H, G	H, G	UH	UH	DA
	(0.03)	(0.05)	(0.06)	(0.07)	(0.11)	(0.09)	(−0.05)	(−0.02)	–

For water hyacinth weight in bracket shows absolute weight. For algae weight in bracket shows specific growth rate mg/L. Values given are the mean values of three replicates

H healthy, *G* green, *YL* yellow leaves, *DP* dead plant, *DA* dead algae, *UH* unhealthy

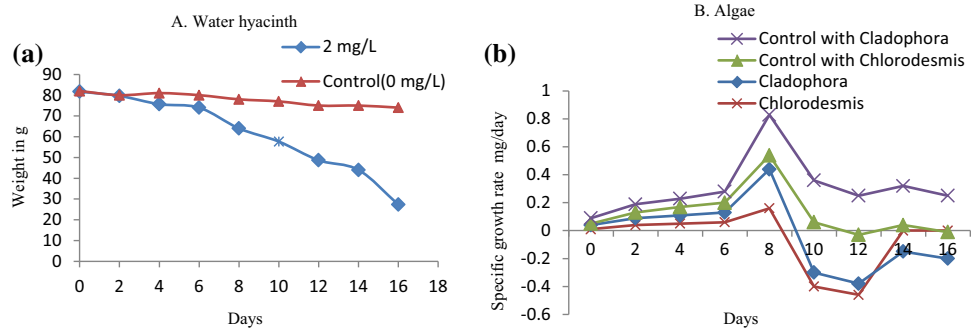

Fig. 1 Growth of aquatic species. **a** Water hyacinth, **b** *Cladophora* sp. and *Chlorodesmis* sp. in 2 mg/L of arsenic-enriched water

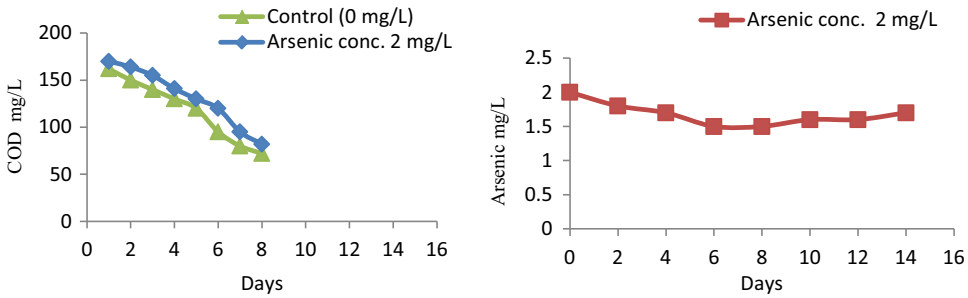

Fig. 2 Changes in water quality with water hyacinth

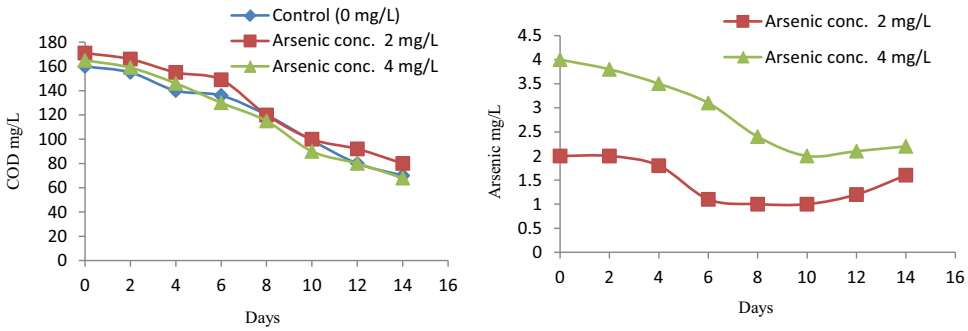

Fig. 3 Changes in water quality with *Chlorodesmis* sp.

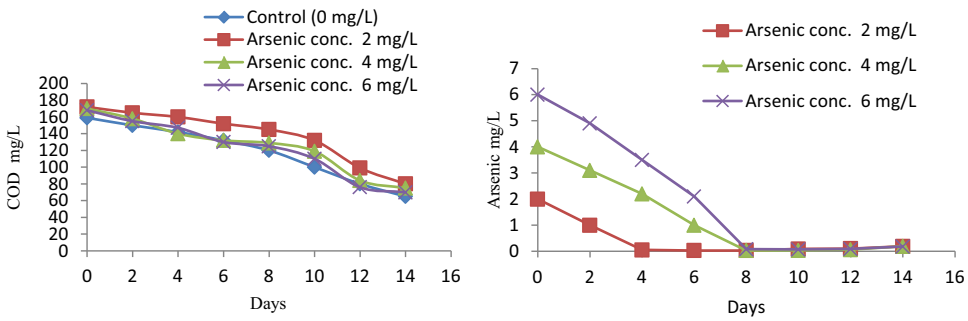

Fig. 4 Changes in water quality with *Cladophora* sp.

Conclusions

It is evident from this study that living algae species were more tolerant to arsenic (III and V) exposure as compared to water hyacinth. *Cladophora* sp. can survive under extreme arsenic conditions and also has high arsenic removal efficiency. It is found that it can bring down arsenic concentration from 6 mg/L to less than 0.1 mg/L during a retention period of 10 days, with pH ranging between 7.2 and 7.5, and under ambient temperature conditions (22−35 °C and incoming solar radiation in range of 3−5.6 kWh/m^2). *Chlorodesmis* sp. can survive up to arsenic concentration of 4 mg/L under similar conditions as of *Cladophora* sp. Water hyacinth is not that effective and survives under low arsenic conditions up to 2 mg/L with a removal efficiency of 20 % only. Specific growth rate of algal species ranged between 0.03 and 0.10 for *Cladophora* sp. and 0.01 and 0.10 for *Chlorodesmis* sp., respectively; whereas, the wet weight of water hyacinth ranged between 26 and 81 gm. COD removal efficiency is also different for each of the species and the values being 50 % for water hyacinth, 50−55 % for *Chlorodesmis* sp., and 55−60 % for *Cladophora* sp., respectively.

Finally, the study confirms that the algae species *Cladophora* sp. is sufficient to remediate arsenic-bearing wastewater and can make it suitable for irrigation. This identified species can therefore, offer a cost-effective solution according to the standards promulgated by Central Pollution Control Board of India (CPCB 1998).

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