Chapter 10

Phytoremediation of Arsenic Contaminated Water

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Additional information is available at the end of the chapter

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Abstract

The present investigation deals with the detoxification of arsenic contaminated water using phytoremediation technique. Three macrophytes *Azolla pinnata*, *Lemna minor*, and *Hydrilla verticillata* were exposed to 1.0 ppm of an arsenic salt (sodium arsenite) separately as well as in combination (*ALH*) for 10 days. The concentration of arsenic in control (wild) macrophytes was below detectable limit. Following exposure, the concentration of arsenic increased steadily in all the plants, and after 10 days, the efficacy of arsenic depletion in phytoremediated media was in the order: *A. pinnata* (88.06%) > *L. minor* (82.56%) > *H. verticillata* (77.53%) and 85.50% when applied in combination (*ALH*). It was found that *A. pinnata* can detoxify the arsenic contaminated water most efficiently.

Keywords: arsenic contamination, bioaccumulation, macromolecular depletion macrophytes, phytoremediation

1. Introduction

In recent years, the areas having arsenic contamination in the ground as well as surface waters are enlarging rapidly in India and its neighboring countries [1–3]. Arsenic is for the most part distributed into the nature in form of either metalloids or chemical compounds, which causes a variety of pathogenic conditions as well as cutaneous and visceral malignancies [4]. Arsenic shows toxicity even at low exposures [5] and causes black foot disease [6]. It is posing great challenge to environmental biologists as well as toxicologist to negotiate the problem. A cost effective technologies are needed to eliminate it from the contaminated water. Phytoremediation is a novel, cost effective and eco-friendly bioremediation technology for environmental cleanup. Bioremediation using macrophytes has been a successful tool to detoxify metal contaminations from variously polluted effluents [7, 8]. Under present evaluation, the arsenic removal competencies from arsenic contaminated water were

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assessed using three widely distributed aquatic macrophytes (*Azolla pinnata, Lemna minor,* and *Hydrilla verticillata*).

2. Materials and methods

The experimental aquatic plants (*A. pinnata, L. minor,* and *H. verticillata*) were collected from the Agrofarm pond of the Banaras Hindu University, Varanasi, India. For experimentation, monoculture of individual plant was prepared at ambient laboratory temperature under natural photoperiods. Prior to phytoremediation, they were rinsed gently with the tap water (having dissolved O_2 6.3 mg L^{-1}, pH 7.2, water hardness 23.2 mg L^{-1} and room temperature 28 ± 3°C, arsenic concentration below detectable limit) to remove debris. They were subsequently acclimated in tap water for a total period of 2 weeks prior to their application in decontamination activity. The test solution was synthesized by adding 1.0 mg of arsenic in 1.0 L of tap water. This concentration (5%) of the LC_{50} value of *Clarias batrachus* [9] was used for toxicity analysis of the arsenic contamination using fish bioassay technique. Ten grams (fresh weight) of each of the macrophyte was transferred to 10 L of the media. For control, same quantities of each of the plants were put into separate aquaria bearing plain tap water. Growths of each of the macrophytes were also evaluated. For each sampling period, separate experimental and control setups were established.

The percentage of removal efficiency of arsenic by aquatic macrophytes was calculated (**Table 1**) by using the following formula:

$$\% \text{ Removal efficiency} = \frac{C_1 - C_2}{C_1} \times 100 \quad (1)$$

where C_1 is the initial concentration of arsenic in the media, and C_2 is the final concentration of arsenic in the media.

Macrophytes	Initial as concentration in media (ppm)	Residual as concentration in media (ppm)	Efficiency (%)
A. pinnata	1.0	0.119 (11.9%)	88.06
L. minor	1.0	0.174 (17.4%)	82.56
H. verticillata	1.0	0.224 (22.4%)	77.53
Mixture of three macrophytes (*ALH*)	1.0	0.145 (14.5%)	85.50

()Denotes percentage of arsenic residue in media after 10 days of treatment.

Table 1. Arsenic removal efficiency of experimental macrophytes from media.

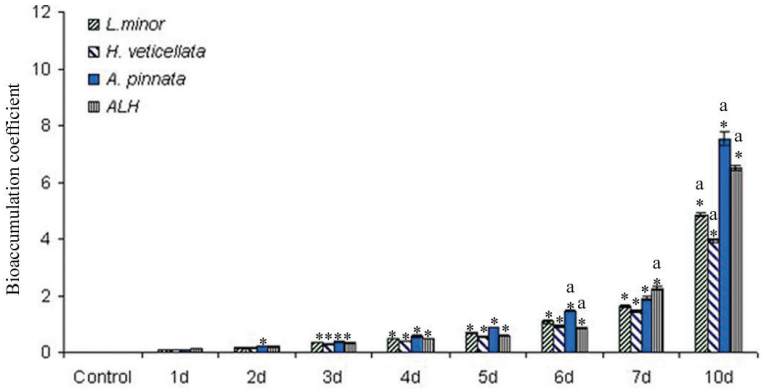

Figure 1. Bioaccumulation coefficient of arsenic at different exposure day. Data are shown as mean ± SEM. The criterion for significant differences set at $p < 0.05$. *Indicates significant differences when compared with 1 day exposed controls. [a]When exposed groups were compared with just previous exposed group.

The bioaccumulation coefficient is defined as the ratio of the concentration of arsenic in the plant and the concentration of residual arsenic in the medium where the plants are growing [10]. The bioaccumulation coefficient was calculated as follows:

$$\text{Bioaccumulation coefficient} = \frac{\text{Arsenic concentration in plant}}{\text{Arsenic concentration in media}} \tag{2}$$

The average bioaccumulation coefficients for the aquatic plants tested here are illustrated in **Figure 1**.

Magnesium ions were analyzed by flame photometer. For chlorophyll estimation, the total chlorophyll was extracted in 80% chilled acetone using Arnon's method [11], prior to observation using a spectrophotometer. The protein content was estimated following Lowry et al. method [12]. Test water was bioremediated with the macrophytes for 10 days. About 1.0 g of each of the plants was collected from experimental setups on different periods (0, 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, and 10th days). For the arsenic analysis, the harvested plants were dried at 80°C. The dried plant materials were digested with acid mixture of HNO_3:$HClO_4$ (5:1 v/v) on a hot plate till a clear solution was obtained. Double distilled water was added to make up the volume to 5 mL. A graphite furnace atomic absorption spectrophotometer (Perkin-Elmer 2380) was used to analyze the arsenic accumulation. All results related to each plant setups were expressed as means followed by standard error of mean. Treatment effects were determined by analyses of variance using the general linear model procedure of the standard statistical analysis system followed by Dunnett's *t*-test at a 5% probability used for post hoc comparisons to separate treatment differences.

3. Results and discussion

Following exposure to the arsenic solution, the macrophytes did not exhibit significant morphological alteration up to 4 days. Then marked deterioration in the physical appearance of

all the three plants was noted in the four experimental setups. The deterioration of the plant health became more extensive after 10 days. However, condition of *A. pinnata* deteriorated early and after 7 days it became obvious. The natures of deterioration in these plants were more or less identical whether treated separately or in combination. The concentration of magnesium ions (Mg^{+2}) in these plants did not show much alteration in all the three plants excepting after 10 days when the alteration was statistically significant. The chlorophyll content of all the three plants showed marked decline following phytoremediation applied singly or in combination of the three macrophytes (**Table 2**). Reduction in the chlorophyll content has been observed in variously phytoremediated plants [13]. However, it has been confirmed that the absorption of heavy metals produces phytotoxic effects on plants resulting in inhibition of chlorophyll synthesis and biomass production that often leads to death [14, 15]. The other reason for depletion of chlorophyll content may be due to impaired uptake of essential elements, damaged photosynthetic components or due to increased chlorophyll activity causing chlorophyll regeneration [16]. The protein concentration of these plants showed progressive depletion, which was statistically significant after 10 days in all the experimental setups. Other toxic metals have also caused reduction in plant protein contents [17].

During phytoremediation, the concentration of arsenic in the media decreased progressively, and the residual arsenic concentrations were 11.93, 17.49, and 22.46 % in *A. pinnata*, *L. minor,* and *H. verticillata,* respectively. The arsenic concentration remained 14.5% in the medium when it was phytoremediated jointly by all the three macrophytes (**Table 1**).

Biomass of all the three macrophytes (*A. pinnata, L. minor,* and *H. verticillata*) increased with exposure period in the order of *A. pinnata* (11.55 ± 0.551) > *H. verticillata* (11.25 ± 0.635) > *L. minor* (11.00 ± 0.550) after 7 days (**Table 3**).

This increase perhaps may partially be due to progressive accumulation of arsenic by these plants. The increase was insignificant after 7 days and onward of exposure. All these plants continued to exhibit detectable arsenic concentrations (**Table 4**). However, after 14 days, they decayed extensively. The bioaccumulation coefficients for the aquatic plants tested in this experiment are shown in **Figure 1**. The results display the bioaccumulation coefficient and illustrate the difference in arsenic accumulation among various macrophyte species. Decrease in the amount of arsenic in the media was due to bioaccumulation of this metalloid by the macrophytes, as reflected by the presence of this metal in the plant tissues (*A. pinnata* accumulated 0.88 mg, *L. minor* 0.82 mg, *H. verticillata* 0.77.5 mg, and 0.85 mg per kg dry wt. of the biomass in the combination of all the three macrophytes) (**Table 4**). The arsenic concentration was below detectable limit in all the three untreated (control) plants. The sum of the arsenic detected in the media after phytoremediation and arsenic absorbed by the plant tissues was quite close to the 1.0 mg L^{-1}, which was the initial concentration prepared for the test solution (**Figures 2** and **3**).

Bharti and Banerjee [13] observed certain degree of difference in sum of the amounts of metals left behind in the phytoremediated coal mine effluent and metal accumulated in the plant tissues after phytoremediation. This was due to their sedimentation, adsorption to the clay particles and organic matters, co-precipitation with secondary minerals, cation-anion exchange, and complexation [7, 18]. In this case, such difference was not noticed because unlike coalmine effluent, the nature and concentration of contaminants in the arsenic solution

Exposure period	Biomolecules	*L. minor*	*H. verticillata*	*A. pinnata*	*ALH*
Control (wild)	Chlorophyll	2.767 ± 0.203[a]	2.533 ± 0.116[a]	3.267 ± 0.392[a]	2.946 ± 0.008[a]
	Mg^{2+}	0.438 ± 0.0014[b]	0.556 ± 0.001[b]	0.519 ± 0.001[b]	0.607 ± 0.004[b]
	Proteins	75.033 ± 1.519[c]	94.833 ± 1.476[c]	82.867 ± 0.284[c]	84.833 ± 1.161[c]
1st day	Chlorophyll	2.633 ± 0.088[a]	2.173 ± 0.039[a]	2.75 ± 0.252[a]	2.836 ± 0.024[a]
	Mg^{2+}	0.434 ± 0.001[b]	0.514 ± 0.001[b]	0.516 ± 0.001[b]	0.584 ± 0.004[b]
	Proteins	70.306 ± 0.454[c]	90.153 ± 0.608[c]	80.42 ± 1.242[c]	80.886 ± 0.315[c]
2nd day	Chlorophyll	2.093 ± 0.064[a]	1.926 ± 0.039[d]	2.65 ± 0.078[a]	2.473 ± 0.041[a]
	Mg^{2+}	0.416 ± 0.001[b]	0.511 ± 0.001[b]	0.507 ± 0.001[b]	0.558 ± 0.007[b]
	Proteins	66.383 ± 1.732[d]	82.013 ± 0.325[e]	72.78 ± 0.015[d]	72.667 ± 0.576[d]
3rd day	Chlorophyll	1.997 ± 0.097[e]	1.786 ± 0.032[d]	1.84 ± 0.041[e]	1.867 ± 0.029[e]
	Mg^{2+}	0.407 ± 0.001[b]	0.492 ± 0.001[b]	0.504 ± 0.001[b]	0.542 ± 0.004[b]
	Proteins	62.356 ± 0.305[f]	76.82 ± 2.061[f]	66.956 ± 2.14[f]	66.903 ± 0.214[f]
4th day	Chlorophyll	1.873 ± 0.0218[e]	1.416 ± 0.088[d]	1.736 ± 0.044[e]	1.773 ± 0.012[e]
	Mg^{2+}	0.402 ± 0.001[b]	0.485 ± 0.001[b]	0.502 ± 0.001[b]	0.5073 ± 0.004[b]
	Proteins	56.946 ± 2.153[g]	70.053 ± 0.913[g]	62.703 ± 2.123[f]	64.623 ± 0.446[f]
5th day	Chlorophyll	1.713 ± 0.027[e]	1.166 ± 0.044[d]	1.573 ± 0.062[e]	1.156 ± 0.024[e]
	Mg^{2+}	0.396 ± 0.001[b]	0.480 ± 0.001[b]	0.496 ± 0.001[b]	0.477 ± 0.001[b]
	Proteins	54.573 ± 1.097[g]	64.403 ± 1.188[h]	57.906 ± 1.166[f]	58.453 ± 1.622[g]
6th day	Chlorophyll	1.503 ± 0.933[e]	0.926 ± 0.039[d]	1.383 ± 0.084[e]	1.306 ± 0.022[e]
	Mg^{2+}	0.378 ± 0.002[b]	0.473 ± 0.001[b]	0.492 ± 0.001[b]	0.455 ± 0.004[b]
	Proteins	48.383 ± 0.661[h]	56.687 ± 1.567[i]	44.38 ± 1.603[g]	50.686 ± 2.391[h]
7th day	Chlorophyll	1.283 ± 0.116[e]	0.796 ± 0.029[d]	1.133 ± 0.060[e]	1.836 ± 0.059[e]
	Mg^{2+}	0.368 ± 0.002[b]	0.465 ± 0.002[b]	0.489 ± 0.001[b]	0.438 ± 0.004[b]
	Proteins	42.233 ± 0.913[i]	48.226 ± 0.597[j]	34.86 ± 0.311[h]	42.576 ± 0.864[i]
10th day	Chlorophyll	0.673 ± 0.092[j]	0.503 ± 0.029[k]	0.693 ± 0.088[i]	0.653 ± 0.057[j]
	Mg^{2+}	0.309 ± 0.023[k]	0.409 ± 0.015[b]	0.471 ± 0.001[b]	0.382 ± 0.004[k]
	Proteins	35.593 ± 1.161[l]	39.156 ± 2.158[l]	28.343 ± 1.472[j]	35.806 ± 1.675[l]

[a–l]Values followed by different consequent alphabetic (a–l) superscript lowercase letters within the same column are significantly different at $p < 0.05$ level according to Dunnett's *t*-test, and the same letter within the column shows insignificant difference between the mean when compared to preceding value.

Table 2. Variation in different biomolecule (chlorophylls, magnesium ions, and proteins) contents (μg g^{-1} fresh wt.) of all the three macrophytes after different periods of exposure to 1.0 ppm of sodium arsenite solution.

were simple. A survey of **Figure 2** clearly shows that uptakes of arsenic in all the macrophytes are time dependent up to 10 days of treatment. **Figure 3** illustrated the relationship between arsenic uptake by the plants and its depletion from the contaminated medium. All the three macrophytes are useful for decontamination of arsenic. This study suggests that *A. pinnata* is

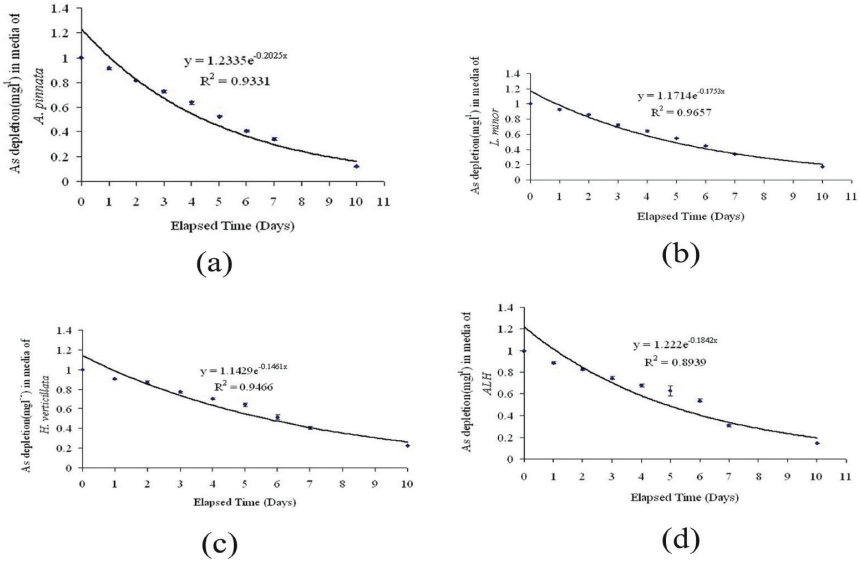

Figure 2. (a-d) Arsenic depletion curve shown by all three plants (*Azolla pinnata*, *Lemna minor* and *Hydrilla verticillata*) separately as well as in their combination (1:1:1) in 10 L of arsenic media (As=1000 μg L^{-1}) for 10 days.

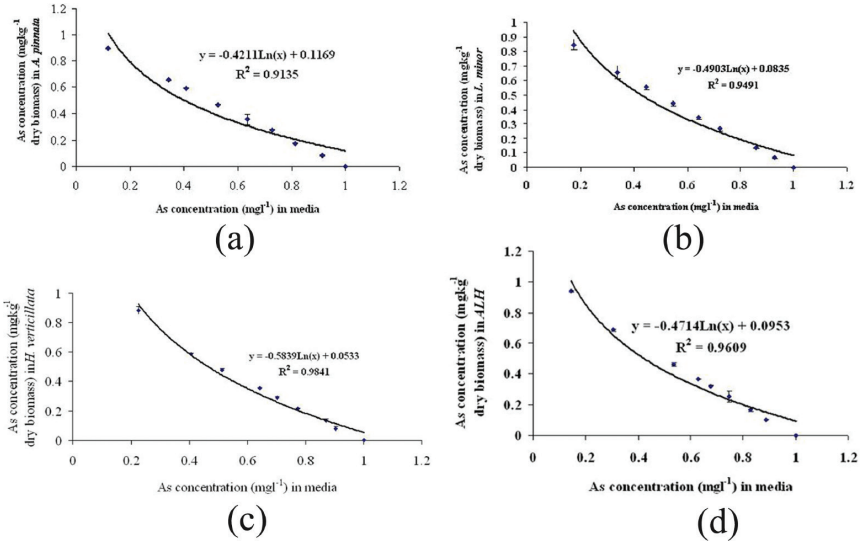

Figure 3. (a–d) Regression curves between arsenic concentration in plant tissues and media of macrophytes (media = grown in arsenic media) at different exposure day.

Macrophytes	Fresh wt. (g) taken at initial day of experiment	Changes of biomass (g) at the end of 7 days of experiment	Changes of biomass (g) at the end of 10 days of experiment
A. pinnata	10.00 ± 0.00[a]	11.55 ± 0.551[a] (+15.5%)	8.95 ± 0.550[b] (−10.5%)
L. minor	10.00 ± 0.00[a]	11.00 ± 0.550[a] (+10%)	9.50 ± 0.635[b] (−5%)
H. verticillata	10.00 ± 0.00[a]	11.25 ± 0.635[a] (+12.5%)	9.65 ± 0.650[b] (−3.5%)

[a, b]Values refer to the mean followed by standard error of mean. Means followed by the same letter in a column were not significantly different at $p < 0.05$.

[()]Denotes percentage change of biomass after 7 and 10 days.

Table 3. The changes of macrophytes fresh biomass during the experiment.

Period of exposure	Macrophytes			
	L. minor	*H. verticillata*	*A. pinnata*	*ALH*
Control (wild)	φ	φ	φ	φ
1st day	0.071 ± 0.004[a]	0.077 ± 0.004[a]	0.084 ± 0.005[a]	0.105 ± 0.001[a]
2nd day	0.134 ± 0.003[a,b]	0.133 ± 0.008[b]	0.176 ± 0.002[a]	0.169 ± 0.009[a]
3rd day	0.267 ± 0.012[b]	0.217 ± 0.002[c]	0.274 ± 0.002[b]	0.253 ± 0.020[b]
4th day	0.346 ± 0.008[b,c]	0.290 ± 0.003[d]	0.353 ± 0.022[b]	0.323 ± 0.001[c]
5th day	0.443 ± 0.012[c,d]	0.353 ± 0.002[d]	0.466 ± 0.001[b,c]	0.367 ± 0.001[d]
6th day	0.553 ± 0.008 [e]	0.476 ± 0.005[e]	0.593 ± 0.001[c,d]	0.462 ± 0.007[e]
7th day	0.657 ± 0.024[f]	0.586 ± 0.001[f]	0.654 ± 0.002 [d,e]	0.687 ± 0.003[f]
10th day	0.823 ± 0.020[g]	0.775 ± 0.005[g]	0.880 ± 0.003[g]	0.853 ± 0.006[g]

[a–g]Values followed by different consequent alphabetic (a–g) superscript lower case letters within the same column are significantly different at $p < 0.05$ level to Dunnett's *t*-test, and the same letter in a column was not significantly different between the mean when compared to just preceding value at $p < 0.05$.

ALH: Mixture of three macrophytes (*L. minor, H. verticillata,* and *A. pinnata*) in equal (1:1:1) ratio.

φBelow detection limit.

Table 4. Arsenic accumulation in different macrophytes at different periods of exposure.

the most efficient plant and can be used singly for this purpose. Phytoremediation beyond 10 days by the same plant will not have additional benefits.

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