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Study of selected aquatic plants as bio-indicator of Arsenic

• **Shweta** • **Bhawana** • **Soghra**
• **Urvashi Sinha**

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Corresponding Author : Urvashi Sinha

Abstract: *In this study species of Eichhornia, Hydrilla, Marsilea and Pistea were exposed to three Arsenic concentrations (1mg/L, 2mg/L and 3mg/L). The selected aquatic plants were screened for potential bio-indicator of Arsenic, by observing the morphological changes and analyzing the effect of Arsenic on chlorophyll (Chl a) content of the leaves and COD of water enriched by Arsenic. Changes were observed in the morphological character of the plant samples as the colour of the plant changed from green to yellow, to brown and death at the end of retention period. Among all the samples lowest chlorophyll content at concentration 3mg/L was estimated as 3.05 mg/L in Eichhornia spp. Highest COD was also observed in Eichhornia as 2.86 mg/L at concentration 3mg/L. Marked changes were also observed in all the species selected. Hence, the selected aquatic plants are effective bio-indicator of Arsenic.*

Keywords*: Phytoremediation, Chemical Oxygen Demand, Total chlorophyll concentration, UV-VIS Spectrophotometer.*

Shweta
B.Sc. III year, Botany (Hons.),
Session : 2016-2019, Patna Women's College,
Patna University, Patna, Bihar, India

Bhawana
B.Sc. III year, Botany (Hons.),
Session : 2016-2019, Patna Women's College,
Patna University, Patna, Bihar, India

Soghra
B.Sc. III year, Botany (Hons.),
Session : 2016-2019, Patna Women's College,
Patna University, Patna, Bihar, India

Urvashi Sinha
Asst. Prof., Department of Botany,
Patna Women's College, Bailey Road,
Patna – 800 001, Bihar, India.
E-mail : .urvashi_vrm@yahoo.co.in

Introduction:

Arsenic is a chemical element which belongs to nitrogen group. In drinking water 50 ppb is considered as the accepted level for Arsenic. In aquatic plants the accepted level for concentration of Arsenic is reported as 0.002-0.25mgml^{-1} (Zhao et al 2010). Plants may indicate the quality of water in a wide variety of ways. Their distribution in morphological character, COD and chlorophyll pigments may be used to detect important feature of the aquatic plants. Aquatic plants have been suggested as bio-indicator to monitor changes in Arsenic level in the aquatic environment. This ability of the plants can be monitored in the laboratory. Aquatic plants must be representative of the area where Arsenic is collected and allow for identification.

The purpose of the present study is to evaluate the use of selected aquatic plants as bio-indicator of Arsenic. The selected aquatic plant species are *Hydrilla, Marsilea, Pistea and Eichhornia.* These aquatic plants are found to remove arsenic ions as reported by Silveira et al (2015).The effect on aquatic plants due to absorption of arsenic can be seen on the chlorophyll content of those plants. As the concentration of Arsenic in water increases the concentration of chlorophyll pigment decreases which is bio-indicator of Arsenic content in water. Change in general appearance of the aquatic plants like colour of the leaf and wilting is also an indicator of high concentration of Arsenic in water. Many results have been documented to show the phyto-remediation ability of the free-floating aquatic plants for nutrient-rich waters (Alvarado et al 2008).

Materials and Methods:

Selected species of *Eichhornia, Marsilea, Hydrilla* and *Pistea* were collected from river Ganges near engineering college ghaat Patna. Plant samples were cleaned with distilled water to remove the attached dirt and soil.

(Lizama et al 2011) was followed to prepare the experimental solutions with Arsenic. It was prepared by adding $Na_2HAsO_4 \cdot 7H_2O$ in a mixture of local tap water and sewage water at 1, 2 and 3 mg/L concentration. Each solution was supplemented with nutrients such as KNO_3 (0.25 gm/L), KH_2PO_4 (0.1 gm/L), $MgSO_4 \cdot 7H_2O$ (0.1 gm/L), and $CaCl_2 \cdot 2H_2O$ (0.1 gm/L) and sewage. The ratio of tap water and sewage were kept as 1:1.

To test the survival of plants in arsenic-enriched water, the tubs were filled with 1000ml (500ml tap water and 500ml sewage water). All the plants were exposed to arsenic concentration of 1, 2 and 3 mg/L for 15 days under ambient sunlight. All experimental solutions were made in triplicate for each of the selected aquatic plant which weigh 50±5 g (wet wt.) growth of the aquatic plants were monitored visually and quantitatively every 5th day.

Method of Ritchie (2006) was followed to estimate the content for chlorophyll a of the samples.

One gram leaves of each plant samples were crushed in mortar and pestle until they were homogenous. 2 ml of 80% acetone and 0.1% sand was added and filtered, Absorbance of the leaf extracts were taken at 663.2 and 646.8nm.

COD of water was analyzed as per standard method. On every 5th day 50 ml of water samples were taken from each tub for water quality analysis.

Chlorophyll (*Chl a*) concentrations (mg/L) was estimated using spectrophotometer at wavelengths two different wavelength 663.2 and 646.8 nm, respectively. Aquatic plants showing survival were monitored for their growth rate at interval of 5 days.

The experiments were performed in similar procedure as used for testing survival rates on 5th, 10th and 15th day (Jasrotia et al 2014).

Harkins (1910) was followed to detect Arsenic to the sample leaf extracts conc. HCl was added and then H_2S gas was passed. Yellow precipitate was obtained proved the presence of Arsenic in the plant samples. Confirmatory test was also done for presence of Arsenic. Sample extracts were taken in a test tube and zinc dust and conc. HNO_3 were added to it. Silver ppt. was obtained that confirmed the presence of Arsenic in the samples.

Results and Discussion:

The study reveals presence of Arsenic in all the plant samples. Selected aquatic plants were healthy and green in colour at the beginning of the

experiment. Leaves turned yellow on 10th day and on 15th day all the sample plants turned brown in colour as shown in Table 1. This result is similar to the result reported earlier by Ingole and Bhole (2003). It was concluded that, by using aquatic plants Arsenic could be effectively removed from waste water when its concentration was less than 10 mg/L.

The effect on aquatic plants due to absorption of Arsenic was observed on the chlorophyll content of those plants. As the concentration of Arsenic in water increased, the amount of chlorophyll pigment decreases in all the plant samples as shown in Table 2. due to the formation of chlorosis in the leaves. Change in colour of leaves was seen in *Pistea spp.* on 5th day which indicated high concentration of Arsenic in water. Many results have been documented to show the phyto-remediation ability of the free-floating aquatic plants for nutrient-rich waters (Fadila et al 2009).

The effect on morphological part of selected aquatic plants was monitored visually and quantitatively on every 5th day. Visual assessment of selected aquatic plant was done by observing changes in the general appearance of the plant such a colour of leaves, stolon, and wilting. Firstly wilting and colour change was seen in *Pistea* on day 5, and then after changes were seen in *Marsilea spp.* followed by *Hydrilla* and *Eichhornia spp.* as shown in Table 1.

The Arsenic removal efficiency by the samples was observed with the decrease in chlorophyll content of aquatic plants. On 5th day all the plants were unhealthy and green except *Pistea* which turned yellow in colour. On 10th day *Eichhornia, Hydrilla and Marsilea* turned yellowish in colour whereas *Pistea spp.* appeared brown in colour and was partially dead.

Table 1. Morphological characters and COD of selected aquatic plants at different concentration of Arsenic

Duration	Plant sample at different concentration of Arsenic (mg/L)	Morphological character	Chemical oxygen demand (mg/L)
5th day	*Eichhornia*		
	1	UH,G	1.01
	2	UH,G	1.26
	3	UH,G	**1.58**
	Hydrilla		
	1	UH,G	1.03
	2	UH,G	1.19
	3	UH,G	1.54
	Marsilea		
	1	UH,G	0.73
	2	UH,G	1.16
	3	UH,G	1.30
	Pistea		
	1	UH,G+YL	0.59
	2	UH,G+YL	0.65
	3	UH,G+YL	0.69
10th day	*Eichhornia*		
	1	UH, YL	1.34
	2	UH, YL	2.16
	3	UH, YL	2.32
	Hydrilla		
	1	UH, YL	1.64
	2	UH, YL	1.91
	3	UH, YL	2.12
	Marsilea		
	1	UH, YL	0.97
	2	UH, YL	1.66
	3	UH, YL	1.85
	Pistea		
	1	DP+BL	1.02
	2	DP+BL	1.33
	3	DP+BL	1.82
15th day	*Eichhornia*		
	1	DP +BL	1.60
	2	DP +BL	2.82
	3	DP +BL	**2.86**
	Hydrilla		
	1	DP +BL	1.42
	2	DP +BL	1.68
	3	DP +BL	2.33
	Marsilea		
	1	DP +BL	1.22
	2	DP +BL	1.64
	3	DP +BL	1.97
	Pistea		
	1	DP +BL	1.19
	2	DP +BL	1.68
	3	DP +BL	1.70

UH- Unhealthy; G- Green; YL- Yellow Leaves; DP- Dead Plant; BL- Brown Leaves

Table 2. Estimation of content of chlorophyll a at different concentration of Arsenic by UV-*VIS* Spectrophotometer

Duration	Plant sample at different concentration of Arsenic (mg/L)	Absorbance at different Wavelength (nm)		Content (mg/L) of chlorophyll a
		646.8	663.2	
5th day	*Eichhornia*			
	1	2.946	2.865	**27.26**
	2	2.713	2.813	26.08
	3	2.992	2.792	24.91
	Hydrilla			
	1	1.266.	1.332	12.22
	2	1.944	2.033	11.51
	3	2.004	2.165	5.64
	Marsilea			
	1	1.713	1.714	15.98
	2	1.685	1.712	14.70
	3	1.684	1.701	13.80
	Pistea			
	1	2.492	2.662	23.97
	2	2.625	2.817	22.09
	3	2.803	2.952	14.60
10th day	*Eichhornia*			
	1	0.219	0.215	12.32
	2	2.400	0.456	7.64
	3	0.652	0.650	6.81
	Hydrilla			
	1	1.364	1.336	18.56
	2	0.639	0.635	17.62
	3	1.191	1.169	11.04
	Marsilea			
	1	0.917	0.913	21.82
	2	0.827	0.815	21.62
	3	0.811	0.808	12.30
	Pistea			
	1	1.830	1.806	19.50
	2	0.288	0.282	16.92
	3	1.526	1.495	16.68
15th day	*Eichhornia*			
	1	1.317	1.300	8.57
	2	0.259	0.255	4.93
	3	0.466	0.460	**3.05**
	Hydrilla			
	1	0.595	0.589	7.52
	2	0.667	0.665	5.87
	3	0.714	0.715	5.61
	Marsilea			
	1	0.917	0.913	8.69
	2	0.827	0.815	8.22
	3	0.811	0.808	7.52
	Pistea			
	1	1.830	1.806	16.68
	2	0.288	0.282	14.33
	3	1.526	1.495	11.64

On 15th day all the plants were completely dead and brownish in appearance as shown in Table 2. The chlorophyll content of 5th day and 15th day, it was found that *Eichhornia* has the highest chlorophyll content at concentration 1mg/L is estimated as 27.26 mg/L and *Hydrilla* has the lowest chlorophyll content at concentration 3mg/L is estimated as 5.64 mg/L, whereas on 15th day *Pistea* has the highest chlorophyll content at concentration 1mg/L is estimated as 16.68 mg/ L and *Eichhornia* has the lowest chlorophyll content at concentration 3mg/L is estimated as 3.05 mg/L as shown in Table 1. The result of work gets support from the work reported earlier by Favas et al (2012).

As shown in Table 2. the level of COD increases after every 5th day with increase in the concentration of Arsenic in water due to cell surface interactions and inter cellular accumulation. On 5th day, it was found that *Eichhornia* has the highest COD at concentration 3mg/L is estimated as 1.58 mg/L and *Pistea* has the lowest COD at concentration 1mg/L is estimated as 0.59 mg/L, whereas on 15th day *Eichhornia* has the highest COD at concentration 3mg/L is estimated as 2.86 mg/L and *Pistea* has the lowest COD at concentration 1mg/L is estimated as 1.19 mg/L.

Conclusion:

It is evident from this study that living selected aquatic plants are bio-indicator of Arsenic. Detection of Arsenic in the plant samples during the retention period of 15 days confirms that the visual morphological changes observed were due to effect of Arsenic. General appearance of the plants, color of leaves, stolon, wilting and drooping of leaves took place as the concentration of Arsenic in plants increased day by day. Reduced concentration of chlorophyll a content in the selected samples suggest the harmful marked effect of Arsenic on the plants. The effect of Arsenic

on total chlorophyll a content justifies the work that the aquatic plants are bio-indicator of Arsenic. All the selected samples functions as bio-indicators but scientifically *Eichhornia spp* was concluded as the most effective bio-indicator of Arsenic. Presence of well developed fibrous roots helps the plant in absorption of As the most. Different COD removal efficiency increases with increase in Arsenic concentration due to cell surface interactions and inter cellular accumulation which suggests that the selected aquatic plants are possible bio- indicators of Arsenic.

In this way the selected aquatic plants are eco-friendly for human being. With the help of some factor we can easily detect the toxicity of Arsenic in the plants. We can also use the COD tested water in the field of irrigation. Save plants, save earth, save the life of human being.

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