ESSENCE - International Journal for Environmental Rehabilitation and Conservation
Volume VIII: No. 1 2017 [36 – 45] [ISSN 0975 - 6272]
[www.essence-journal.com]

Effect of arsenic on plant growth and remediation of arsenic by Cyanobacterial species

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Received: March 12, 2017 | **Accepted**: April 29, 2017 | **Online**: June 30, 2017

Abstract

Arsenic is being a toxic metal for all living form. The uptake of arsenic is not identical for all plant varieties, its extents depends upon the plant species. The three species of Cyanobacteria namely, *Anabaena*, *Nostoc* and *Leptolyngbya* were grown in BG11 medium supplemented with soil and the effect of arsenic (250µg/L) on plant growth of marigold were observed. The parameters of the plant growth namely, height of the plant in cm., average leaf length in cm., number of branches and perimeter of the stem in cm. were considered for overall growth of the plant. The residual arsenic is 24.4 µg suggesting thereby that *Anabaena* must have removed considerable arsenic from the soil. In case of *Nostoc* and *Leptolyngbya* the residual value was found to be 20.4 µg, 37.6 µg respectively. Thus all the three species removed arsenic from the soil.

Keywords: Arsenic | Cyanobacteria | plant growth | remediation of arsenic

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Introduction

Arsenic is widely distributed toxic element that is found in the Earth crusts. This metal has a tendency of contaminating underground water resulting in its re-distribution not only along multidimensions of Earth but also among the plants and animals. The presence of arsenic in drinking water thus becomes a serious water pollution problem in many countries including India and Bangladesh. Arsenic pollution is a serious problem which not only occurs through contaminated drinking water but also from human solid food chain when the crops and fodder become contaminated with arsenic. The Indian subcontinent is primarily facing this problem where contaminated drinking water acts as a principle source of arsenic pollution (Nordstrom, 2002). The metalloid arsenic can also enter into the farming systems through natural geochemical processes partly due to many agencies namely, use of arsenic based pesticides, mining operations, use of arsenic containing groundwater for irrigation and the use of municipal solid waste for fertilization (Smedley and Kinniburgh, 2002; Meharg *et al*., 2009). Among the various food crops, rice grain is significantly important as it acts as a major source of arsenic contaminated crop. Apart from rice, beet-root, potato, orchaids, blue-berry and tobacco have been reported to be containing sufficiently high level of arsenic. Apart from these, peas, beans, soya beans, cotton have also been reported to be contaminated with arsenic (Walash *et al*., 1977).

In the recent times, the arsenic content is significantly alleviated in the soil, primarily due to the use of pesticides containing arsenicals (Adriano, 2001). Most of the plants show uptake of arsenic from the soil and at times as such gets accumulated within certain plants named as hyperaccumulaters (Brooks, 1998). The uptake of arsenic is not identical for all plant varieties. The extent of arsenic uptake depends upon plant species (Matschullat, 2000). Many other factors also decide the extent of arsenic uptake *e.g.* concentration of arsenic in soil, the basic properties of the soil and even other ions present in the soil affect the arsenic uptake (Jiang and Singh, 1994, Khattak, 1991). The objectives of the present study were

- To study the effect of arsenic on the overall growth of marigold plant.
- The effect of *Cyanobacteria* on the arsenic uptake by the plants.

Materials and Methods

Preparation of soil samples for experimentation

5 kg of garden soil was purchased from nursery. The soil pH, moisture content and the colour were recorded. After thorough mixing the whole lot of the soil was sterilized to remove the rhizosphere bacteria and other contaminants. The soil was taken in sterilizable cans and autoclaved at 15 pounds/sq. inch pressure resulting in 121^0C for 15 min. After the sterilization the soil sample was allowed to cool down and mixed with a vitamin supplement A to $Z_{NS\ New}$. After thorough mixing 500gm of soil was placed in pots. Numbers of pots were prepared and in each set one was kept for control and the other pots were designated for specific experiments.

Estimation of arsenic

Throughout all the experiments mili-Q water was used for carrying out the experiments. The standard solution of the arsenic was prepared by dissolving sodium arsenate in mili-Q water to prepared according to need of the experiment. Whenever the sample was collected, they were filtered through Wattman's filter paper no. 42 and the filtrate was maintained at pH 2 by adding nitric acid and all the samples were preserved at 4^oC for further analysis. The arsenic was estimated by Atomic Absorption Spectroscopy (AAS) where standardization was done at the arsenic content of 50- 250µg/L. Throughout the experiment the arsenic was represented in µg/L.

Results and discussion

Effect of Cyanobacterial supplementation in the soil on the growth of marigold

Cyanobacteria can be divided into nitrogen fixing and non nitrogen fixing groups, out of the three cultures that we have, nitrogen fixing *Anabaena*, *Nostoc* whereas *Leptolyngbya* is a non nitrogen fixing *Cyanobacteria*. Marigold plants were obtained from nursery and were planted in the pots. For each culture duplicates set were prepared. One set of pot was kept as control and in the other set cultures were added with approximately MLSS of 1000 mg/L. The cultures were filtered off and the moist cultures mass was thoroughly mixed with the soil in the pot. Water was sprinkled on each pot on daily basis with approximate 150- 200 ml of water every day. The height of the plant, average leaf length, number of branches and perimeter of the stem were measured for control as well as experimental set. These parameters were monitored every 5 days for a period of 25 days. The average of the two sets were calculated and the results are shown in Table 1, 2 and 3 and photographs 1, 2 and 3

Effect of arsenic on growth of marigold

Two sets of pots were prepared in duplicates. One set of pot was kept as control and the other pot was kept as experimental. Both the sets were planted with marigold. In the experimental set 250µg arsenic was added in the soil and mixed well. Both the sets were watered as described earlier. The height of the plant, average leaf length, number of branches and perimeter of the stem were measured. Initially the experiment was set for 25 days. All the plants were exposed to arsenic showed severe degeneration of leaves and the plants got dried up and seem as if they are burned. The results are shown in Table 4 and photograph 4

Effect of *Cyanobacteria* on the remediation of arsenic

Pots were prepared as described earlier in duplicates. They were divided into two sets, one for control and other for experimental. Pots were then planted with marigold. In the experimental set 250µg of arsenic was mixed and in one set 1000mg of *Anabaena,* in the other set 1000mg of *Nostoc* and in the third set 1000mg of *Leptolyngbya* were added. All the plants were sprinkled with water as described earlier. The height of the plant, average leaf length, number of branches and perimeter of the stem were measured for 25 days. After 25 days the residual arsenic in the soil was determined. The results are shown in table 5, 6 and 7 and in the photograph 5

Arsenic is well known for its toxic effects on plants and *Anabaena* and *Nostoc* being nitrogen fixing organisms are good nitrogen providing species in the rhizosphere. However, *Leptolynbya* being non nitrogen fixer may not be that much useful for plant growth when present in the rhizosphere. Four parameters of the plant growth namely, height of the plant in cm., average leaf length in cm., number of branches and perimeter of the stem in cm. were considered for overall growth of the plant. As can be seen from table 1, the supplementation of *Anabaena* in the soil resulted in significant increase in growth. The height of the plant increased by 134.7% whereas that of growth in the control showed only 36.66% increase. Similarly, the average leaf length also showed increase to 85% when supplemented with *Anabaena* in comparison to 64.28% in control without supplementation. Since the experiment was done for only 25 days therefore there was no significant change in the number of branches. However the lateral growth increased as the perimeter of the stem increased by 88.8% in comparison to control showing increase of 27.27%.

Nostoc showed little bit different result in comparison to *Anabaena*. As can be seen from table 1.2, average leaf length did not change much in control and experiment. Similarly the lateral growth in terms of perimeter of stem also showed only 6% increase in the experimental in comparison to control. Height of the plant did increase in the experimental in comparison to control but the numbers of branches were negatively affected in the experimental. As can be seen from table 1.3 *Leptolynbya* were not able to influence the growth of marigold plant as the control showed much better growth in comparison to experimental.

When the potted plants were supplemented with arsenic, the marigold plants experienced severe toxic effects as can be seen from Table 4 and photograph 4. When the marigold plants were grown in presence of Cyanobacterial species and arsenic at 250µg/L, the hypothesis behind this experiment was that the Cyanobacterial species can either adsorb or accumulate arsenic thereby reducing the effect of arsenic toxicity to the plants. As can be seen from Table 5, 6 and 7 that supplementation of *Anabaena*, *Nostoc* and *Leptolyngbya* affected the growth parameters and the growth in experimental was found to be much better. In case of experimental when the arsenic present in the soil were estimated the residual arsenic was very low as compare to supplemented value. The residual arsenic is 24.4 µg suggesting thereby that *Anabaena* must have removed considerable arsenic from the soil (Table 5). In case of *Nostoc* the residual value was found to be 20.4 µg and that of *Leptolyngbya* was found to be 37.6 µg in the soil (Table 6, 7). Thus all the three species removed arsenic from the soil.

S. No.	Parameters	Control			Experimental		
		Initial	Final	% increase	Initial	Final	% increase
1.	Height of the plant (in cm.)	6±1.2	8.2±0.6	36.66	2.3±0.1	5.4±0.3	134.7
2.	Average leaf length (in cm.)	1.4±0.1	2.3±0.2	64.28	2±0.2	3.7±0.1	85
3.	Number of branches	8	8	0	6	6	0
4.	Perimeter of stem (in cm.)	1.1±0.1	1.4±0.2	27.27	0.9±0.05	1.7±0.1	88.8

Table 1: Effect of *Anabaena* on growth of marigold

S. No.	Parameters	Control			Experimental		
		Initial	Final	% increase	Initial	Final	% increase
1.	Height of the plant (in cm.)	5.5±0.2	7.2±0.1	30.90	10.5±0.5	16.5±0.2	57.14
2.	Average leaf length (in cm.)	3.0±0.1	3.6±0.1	20	3.1±0.05	3.7±0.2	19.35
3.	Number of branches	6	10	66.66	8	12	50
4.	Perimeter of stem (in cm.)	1.5±0.1	1.8±0.2	20	1.5±0.1	1.9±0.1	26.66

Table 2: Effect of *Nostoc* on growth of marigold

S. No.	Parameters	Control			Experimental		
		Initial	Final	% increase	Initial	Final	% increase
1.	Height of the plant (in cm.)	6.5±0.2	8±0.1	23.07	9±0.3	9.5±0.2	5.55
2.	Average leaf length (in cm.)	3.0±0.1	3.3±0.2	10	2.5±0.1	2.7±0.05	8
3.	Number of branches	8	8	0	8	8	0
4.	Perimeter of stem (in cm.)	1±0.1	2±0.1	100	1.2±0.1	1.6±0.1	33.33

Table 3: Effect of *Leptolyngbya* on growth of marigold

S. No.	Parameters	Control			Experimental		
		Initial	Final	% increase	Initial	Final	% increase
1.	Height of the plant (in cm.)	6.5±1	8.4±0.2		5±0.1	Dried/ death in 5 days.	
2.	Average leaf length (in cm.)	3.2±0.1	3.9±0.1		2.2±0.1	Dried/ death in 5 days	
3.	Number of branches	8	8	0	6	Dried/ death in 5 days	
4.	Perimeter of stem (in cm.)	1.1±0.05	1.3±0.1		0.9±0.1	Dried/ death in 5 days	

Table 4: Effect of arsenic on growth of marigold

S. No.	Parameters	Control			Experimental			µg of arsenic left in soil
		Initial	Final	% increase	Initial	Final	% increase	
1.	Height of the plant (in cm.)	6±0.2	8.2±0.2	36.66	5±0.1	8.5±0.3	70	24.4
2.	Average leaf length (in cm.)	3.2±0.1	3.4±0.1	6.25	2.8±0.1	4.1±0.3	46.42	
3.	Number of branches	8	8	0	6	7	16.66	
4.	Perimeter of stem (in cm.)	1.2±0.1	1.3±0.1	18.18	1.3±0.1	2.4±0.1	84.61	

Table 5: Effect of *Anabaena* on the remediation of arsenic

S. No.	Parameters	Control			Experimental			µg of arsenic left in soil
		Initial	Final	% increase	Initial	Final	% increase	20.4
1.	Height of the plant (in cm.)	5.5±0.2	7.2±0.1	30.90	10.5±0.1	11.7±0.2	11.42	
2.	Average leaf length (in cm.)	2.9±0.6	3.5±0.4	20.68	3.5±0.3	4.1±0.2	17.14	
3.	Number of branches	10	10	0	12	12	0	
4.	Perimeter of stem (in cm.)	1.2±0.1	1.8±0.2	50	1.5±0.1	2±0.1	33.33	

Table 6: Effect of *Nostoc* on the remediation of arsenic

S. No.	Parameters	Control			Experimental			µg of arsenic left in soil
		Initial	Final	% increase	Initial	Final	% increase	
1.	Height of the plant (in cm.)	5.1±0.1	7.8±0.1	52.94	9±0.2	10.4±0.1	15.55	37.6
2.	Average leaf length (in cm.)	2.5±0.2	3.3±0.1	32	2.6±0.1	3.4±0.1	30.76	
3.	Number of branches	8	8	0	12	12	0	
4.	Perimeter of stem (in cm.)	1±0.1	2±0.2	100	1.4±0.1	1.9±0.2	35.71	

Table 7: Effect of *Leptolyngbya* on the remediation of arsenic

I) Control **II) Experimental**

Photographs 1 - *Anabaena* supplementation in the soil on the growth of marigold

I) Control **II) Experimental**

Photographs 2 – *Nostoc* supplementation in the soil on the growth of marigold

I) Control **II) Experimental**

Photographs 3 –*Leptolyngbya* supplementation in the soil on the growth of marigold

Photograph 4- Marigold plant exposed to arsenic seems dried and burned

Photograph 5- *Anabaena* shows budding stage in the remediation of arsenic

Conclusion

The residual arsenic is 24.4 µg suggest that *Anabaena* must have removed considerable arsenic from the soil. In case of *Nostoc* the residual value was found to be 20.4 µg and that of *Leptolyngbya* was found to be 37.6 µg in the soil. Thus, all the three species removed arsenic from the soil.

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