Journal of Scientific Enquiry
Research Article *Vol:2(2), Pages:87-91*

Effect of arsenic on growth and cellular biochemistry of *Rhizoclonium fontinale* Kützing

Dr. Panchali Bhattacharya[1*]

[1]Department of Botany, Balarampur College, Purulia

Received:14. 11. 22; Accepted: 15.12.2022; Published Online:31.12.2022

Abstract

Population growth and increased technological activity have expedited the addition of several toxic contaminants, particularly metal ions, to the surrounding environment. Due to their mobilisation, transit, and deposition in many aquatic and terrestrial ecosystems, these contaminants become harmful. Not only is the removal of these dangerous metals and metalloids necessary for the environment, but a safer removal procedure is urgently required. Phytoremediation is the most secure method for removing/stabilizing these hazardous environmental contaminants. Arsenic pollution in West Bengal is a serious concern that needs to be addressed. In this study, the effect of arsenic on the green alga *Rhizoclonium fontinale* was experimented with to determine whether it can be an agent for phycoremediation. It was found that, in *R. fontinale,* the biochemical parameters like chlorophyll, carotenoid, and carbohydrate contents increased with time suggesting survival of the organism in high concentrations of arsenic.

Keywords: Arsenic, *Rhizoclonium,* bioremediation

1. Introduction

Arsenic pollution in groundwater is a persistent concern for the 21st century in several areas of the world, with Bangladesh and West Bengal having the greatest populations at danger (India). According to the research of the School of Environmental studies at Jadavpur University [1], the maximum content of lead in drinking water in West Bengal is 3,880 g/lt and in Bangladesh, it is 4,730 g/lt. Not only in West Bengal and Bangladesh but also in the Ron Phibum area of southern Thailand [2], soil and water are extremely polluted with arsenic.

Arsenic in the environment may have disastrous effects on human health and is a major public health problem today. Arsenic is a proven carcinogen and mutagen that poses grave dangers to human and animal health. Chronic exposure to arsenic in drinking water has been associated with adverse health consequences, including skin and internal organ malignancies. Furthermore, studies have demonstrated a tight relationship between breathed and ingested arsenic and cancer rates. The link between arsenic exposure and cancers of the skin, liver, respiratory system, and gastrointestinal tract is widely known. The U.S. Environmental Protection Agency has classed certain arsenic compounds as Class-A human carcinogens. Consequently, the impact of arsenic on human health is a global concern.

Consideration has been given to biomonitoring and phytoremediation of arsenic toxicity to address the serious harm caused by arsenic. It can give cost-effective and visually beautiful methods for removing hazardous metals from polluted environments. The plants can quickly collect the metal to high concentrations and maintain viability over the accumulation phase. These plants are known as hyperaccumulators. The kind of arsenic absorbed by hyperaccumulators is crucial for identifying their suitability to remediate polluted areas, as the arsenic-rich plant itself will ultimately need to be treated. There are several instances of hyperaccumulator algae in relation to the biomonitoring and phytoremediation of arsenic as a phytochelator for aquatic systems.

In the present study, the potential of green filamentous microalgae *Rhizoclonium fontinale* for biomonitoring and phytoremediation of hazardous arsenic in the form of sodium arsenate ($Na_2HAsO_4,7H_2O$) was evaluated. This study examines the effect of the poisonous metalloid on algal cells and its influence on biochemical parameters.

2. Material and Methods

Culturing of the algae used:

*Electronic mail: panchali17@gmail.com

 DOI: https://doi.org/10.54280/jse.222207

The freshwater alga *Rhizoclonium fontinale* was collected from Ballygunge Science College Campus, Kolkata. After collection, the algal biomass was first washed in running tap water for about half an hour, treated with an antibiotic solution, and then it was washed with distilled water.

The alga was cultured the by tank culturing method. About 5 lt. of BBM (Bold Basal Medium) [3] was prepared using tap water. The alga was inoculated and then the tub was covered carefully with a fine net. For well maintenance of the algae, about 2 lt. media was removed and fresh media was added to it at 7 days interval.

Preparation of experimental media:
BBM solution was prepared by using only NaCl, $NaNO_3$, potassium dihydrogen phosphate (KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4) in case of $+PO_4$ medium, as other salts of BBM media get precipitated with arsenate solution. 2.5 gm. Of *R. fontinale* filaments were inoculated in 1ppm and 5ppm of Sodium arsenate salt concentrations for 28 days. The biochemical tests for chlorophyll, carotenoids and carbohydrate were performed with the alga grown in control and treated medium (BBM) at different days intervals to estimate the toxic effects of arsenic.

Determination of accumulation of arsenic by atomic absorption spectrophotometric method:
0.5 gm of dried samples were accurately weighed and prepared for analysis. The digestion procedure consisted of the addition of 5 ml concentrated HCl with swirling in a beaker. About 5ml volume of 30% v/v H_2O_2 was added slowly and the reaction allowed to proceed. This addition of H_2O_2 was repeated and when the reaction had subsided, the digestion mixture was heated on a hot plate. After cooling, 2ml of concentrated HNO_3 was added until the solution was light straw coloured. After filtration, the digested mixture was made upto 25ml. This solution was used for the determination of Arsenic by atomic absorption spectrophotometric method.

Total chlorophyll estimation:
1 gm of fresh algal tissue was taken and grinded in mortar and pestle with acetone. Extraction was done till the algal tissue turns into a colourless mass and the volume was measured. The mixture was centrifuged, and the supernatant was collected. Absorption was measured at 645nm and 663nm.

Total carotenoid estimation:
1 gm of fresh weight of algal tissue was taken and was extracted with 10.0 ml. acetone in mortar and pestle, and then after centrifugation, the supernatant was taken and kept overnight in dark at 4°C. For Saponification, crude pigment extract was taken. It was evaporated to dryness keeping in dark. The residue was dissolved in small volume of ether and equal volume of 10 % methanolic KOH solution was added. It was again kept for 2-3 hrs. at room temperature for saponification in dark. Then it was diluted with 3 volume of water (containing 2.5% NaCl) and 10 ml petroleum ether was added in a separating funnel and shaken gently allowing it to stand until two phases were distinct. The carotenoids remained in the upper phase (ether) which was separated from aqueous phase. The ether layer was extracted several times with more ether and the combined extracts were washed 3 times with more water to remove alkali and methanol. The ether extract was then dried over anhydrous Na_2SO_4 (5-10 gm/100 ml extract) and filtered with sintered glass funnel. The dried extract was again washed with fresh dry ether and transferred to a small 25 ml conical flask. This was then dried under a stream of N_2. Readings were taken at 450nm. for total carotenoids.

Carbohydrate estimation by Anthrone method:
50 mg of the sample was taken into a boiling tube and hydrolyzed by keeping it in a boiling water bath for 3hrs with 5 ml of 2.5(N) HCL and cooled to room temperature. Sodium carbonate was added until the effervescence ceased. The volume was made up to 100 ml and centrifuged. The supernatant was collected and 0.5 and 1ml aliquots were taken for analysis.

The standards were prepared by taking 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 ml of the working standards. Zero served as blank. The volume was made up to 10 ml in all the tubes including the sample tubes by adding distilled water. Then 1 ml from each sample was taken and 4 ml of anthrone reagent was added. These were then heated for 8 minutes in a boiling water bath. After cooling rapidly, green

and dark green colour readings at 630 nm were taken.

3. Results

Atomic Absorption results indicated that arsenic uptake was almost 5 times more in Sodium arsenate-treated alga than in arsenite-treated alga in 3hrs of exposure to 0.1ppm arsenic (Fig.- 1). The alga absorbed 7µg/gm of arsenate and 1.5 µg/gm of arsenite. Arsenic content in arsenite-treated alga was even lower than the control set indicating the presence of arsenic in the natural habitat. The alga absorbed 7µg/gm of arsenate and 1.5 µg/gm of arsenite.

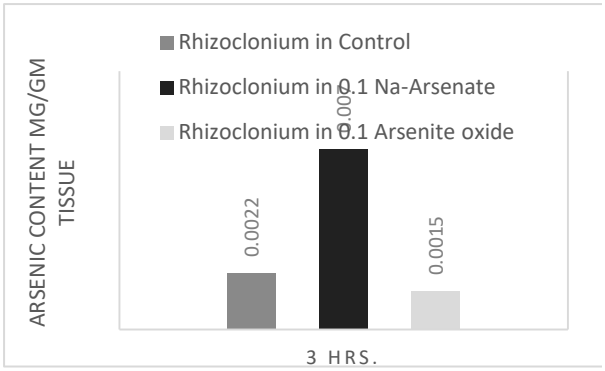

Fig-1: Showing variation in Arsenic content in *Rhizoclonium*

There was a gradual increase in total chlorophyll content in Na-Arsenate treated samples as compared to the control set. Chlorophyll content attended maximum at 5ppm Na-Arsenate at 28 days indicating increased photosynthetic activity (Fig.- 2).

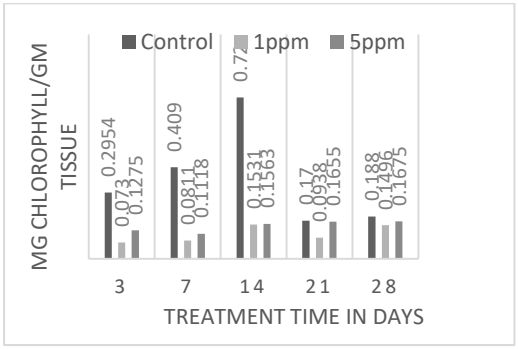

Fig-2: Showing variation in mg-Chlorophyll/gm tissue in *Rhizoclonium*

There was a gradual decrease in carotenoid content in the control set from 0 to 28 days. Whereas there was a gradual increase in the carotenoid content in the Na-Arsenate treated samples. In the control, set shielding of light by carotenoid pigments was much necessary when the algal material was brought from outdoor tank culture to flask culture condition, so its content was reduced. In the treated samples carotenoid content increased with time (Fig.- 3).

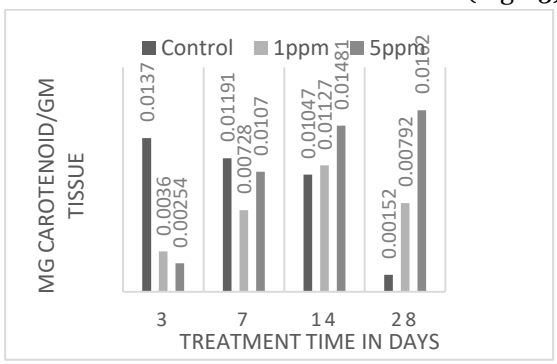

Fig-3: Showing variation in mg-Carotenoid/gm tissue in *Rhizoclonium*

Carbohydrate content in the control did not show much variation over the period of 28 days of control set whereas in treated samples carbohydrate content gradually increased attaining maximum at 5 ppm Na-Arsenate on 28th day (Fig-4).

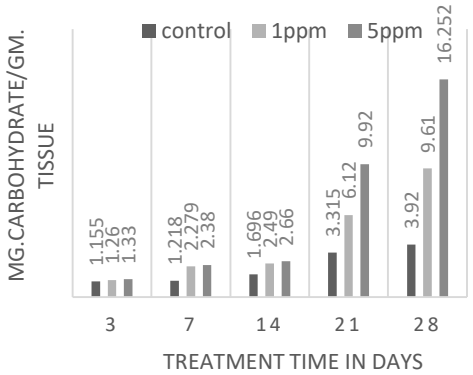

Fig-4: Showing variation in mg-Carbohydrate/gm tissue in *Rhizoclonium*

4. Discussion

As soon as inorganic stressors enter cells, ions may bind to internal components or precipitate [4]. Metal activity influences biological macromolecules and enzymes with proper functional groups or metal co-factor. In *Ankistrodesmus falcatus*, the distribution of Sn was about 85% in the cellular polysaccharide fraction, 15% in the protein fraction, and 0.2% in the lipid and low molecular weight metabolite fraction [5]. Metals can be detoxified by accumulating in polyphosphate bodies and intracellular metal-binding proteins of BGA and eukaryotic algae [6] and within the vacuoles of

some eukaryotic algae [4][7]. Metal accumulation may harm cellular ultrastructure [8][9].

The presence of one inorganic chemical can alter the distribution of other inorganic chemicals inside biological components. For instance, Cadmium and Chromium mixes altered each other's concentrations and distributions in the membrane cell wall, soluble, and other fractions of Chlorella ellipsoid cells [10].

Low degrees of stress tend to increase rather than inhibit responses such as growth rate. Beaumont and Newman [11] discovered that cultures of *Pavlova hetheri* treated to 0.1 g/liter exhibited improved growth. Low concentrations of Cd, Pb, and Ni promoted the growth of three green algae [12]. This may be the result of an increase in enzyme activity or a rise in the concentration of free trace metal ions as a result of ion exchange between Cd, Pb or Ni and the EDTA chelators in the growth medium.

Plate-1: Akinete formation in *R. fontinale*

In our study, it was found that that arsenate had little effect on the chlorophyll content in biosynthetic process. Carotenoid content also increased, which supports the view that in long term exposure of algae to arsenate, the carotenoid acts as an anti-oxidizing agent to reduce the stressed condition. An increase in carbohydrate content was seen. This was due to cell wall thickening and akinete formation, which denotes the development of a physical barrier to resist the entry of arsenate (Plate-1). So, from the study, we found that arsenic treatment increased all the biochemical parameters in *R. fontinale* with very little effect on its growth. So, for this reason, *Rhizoclonium fontinale* can be considered as a biomonitoring or phycoremediation agent for arsenic contamination.

Acknowledgment: The author thanks B.C.K.V, Mohanpur, India for providing AAS for arsenic estimation and also the Department of Science and Technology, Government of West Bengal, India for financial support.

References

[1] M.M. Rahman, J. Ng, and R. Naidu, Chronic exposure of arsenic via drinking water and its adverse health impacts on humans, Environmental geochemistry and health. 31 Suppl 1. 189-200, **(2009)** https://doi.org/10.1007/s10653-008-9235-0.

[2] T. Williams, B. Rawlins, B. Smith *et al.,* In-Vitro Determination of Arsenic Bioavailability in Contaminated Soil and Mineral Beneficiation Waste from Ron Phibun, Southern Thailand: A Basis for Improved Human Risk Assessment, Environmental Geochemistry and Health, 20: 169–177, **(1998)** https://doi.org/10.1023/A:1006545817478

[3] H.C. Bold, The cultivation of algae, Bot. Review, 8: 69–138, **(1942).**

[4] G.M. Gadd, Accumulation of metals by Microorganisms and algae, on "*Biotechnology*" edited by H.J. Rehm, VCH, Weinheim, 60: 401-434, **(1988).**

[5] P.T.S. Wong, R.J. Maguire, Y.K. Chau and O. Kramar, Uptake and Accumulation of Inorganic Tin by a Freshwater Alga, *Ankistrodesmus falcatus*, Canadian Journal of Fisheries and Aquatic Sciences, 41 (11): 1570-1574, **(1984)** https://doi.org/10.1139/f84-195

[6] W.X. Zhang and V. Majidi, Monitoring the cellular response of *Stichococcus baccillaus* to expose of several different metals using in vivo P-31, NMR and other spectroscopic technique, Environ Sci. Technol, 28: 1577-1581, **(1994).**

[7] G.W. Garnham, G.A. Codd and G.M. Gadd, Kinetics of uptake and intracellular location of cobalt, manganese and zinc in the estuarine green alga *Chlorella salina*, Appl Microbiol Biotechnol 37: 270–276, **(1992)** https://doi.org/10.1007/BF00178183

[8] P. Prevot and M.O. Soyer- Gobilland, Combined action of Cadmium and Selenium on two marine dinoflagellates in culture, *Prorocentrum micans* Eurbg and *Crypthecodinium cohnii* Biecheler. J Protozool, 33: 42-47. **(1986).**

[9] M. Romeo, M. Gnassia-Barelli, C. Karez, S. Puiseux-Dao, Cadmium uptake by a phytoplankton species in the presence of different inhibitors, Toxicological & Environmental Chemistry. 22: 97-100, **(1989)** https://doi.org/10.1080/02772248909357429.

[10] H. Okamura and I. Aoyama, Interactive toxic effect and distribution of heavy metals in phytoplankton, Environ. Toxicol. Water Qual, 9: 7-15, **(1994).**

[11] A.R. Beumont and P.B. Neuman, Low levels of tributyl tin reduce growth of marine micro-algae, Mar. Pollut. Bulletin, 17: 457-461, **(1986).**

[12] P.V. Devi Prasad and P.S.D. Devi Prasad, Effect of Cadmium, Lead and Nickel on three freshwater green algae. Water air soil Pollution, 17: 263-268, **(1982).**