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Effect of arsenic on the growth and physiology of *Chaetoceros calcitrans* isolated from Cochin backwaters

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Original Article

Abstract

Arsenic pollution is a major problem in the aquatic environment. In the present investigation, it has been found that micro algae can sequester heavy metals from water. The oxidation state of the metal play an important role in metal uptake capacity. Bioassay study was carried out using the diatom, *Chaetoceros calcitrans* isolated from Cochin backwaters and its growth phase was determined. In the case of estuarine forms, salinity play an important role in the metal uptake and growth. In Cochin backwaters *C. calcitrans* is found to be dominant during the post monsoon period when the salinity ranged from 26 to 35 ppt. High salinity stimulates the metal uptake in *C. calcitrans*. The effect of different concentrations of Arsenic on the growth, productivity, pigments, proline and protein content were studied. It was observed that Arsenic was found to be stimulating the growth upto 5 ppm concentration and was toxic above 10 ppm. Even though an increase in biomass was observed due to initial metal uptake, gradually toxic effect was noticed in the metabolic activity of the cell. This was reflected in the end products of photosynthesis and different pigments. This pilot study can be used as a specific application in environmental biotechnology for the management of Arsenic pollution at low intensity by phyco remediation.

Keywords: *Arsenic, Chaetoceros calcitrans, protein and proline production, physiological parameters, Cochin backwaters.*

Introduction

The presence of heavy metals in the environment is of major concern because of their toxicity, bio-magnification and threat to human life and environment (Igwe and Abia, 2003; Harsfall and Spiff, 2005). Once heavy metals are accumulated by aquatic organisms, they can be transferred to higher levels of food chain. Carnivores at the top of the food chain including human, obtain most of their heavy metals from the aquatic or terrestrial ecosystems by way of their food. So there exists the potential for considerable biomagnifications (Mance, 1987; Langston, 1990).

Biological removal of heavy metals is one of the most promising technologies involved in the removal of toxic metals from the industrial waste streams and natural waters (Brahmbhatt and Rinku, 2012). Many microbial species such as bacteria, fungi, yeast and algae are known to be capable of adsorbing heavy metals on their surface or accumulating within their structure (Beech and Sunner, 2004). It is well known that algae are potentially good candidates for bio sorbent development and an extensive body of literature deals with metal removal by various algae (Wilde and Benemann, 1993).

Test species *Chaetoceros calcitrans* is a marine diatom isolated from

Cochin backwaters and belongs to the Class- Bacillariophyceae. Structurally it is a pennate diatom. At the basal portion, a pair of long horn like hairs are arising from the poles of the elliptical valves. This helps them to aggregate together in a common mass of mucilage. The horn like projection can be seen only by electron microscope. *Chaetoceros* produce heavily silicified resting cells that are often preserved in sediments. Because of high growth rates and high concentrations of lipids, *Chaetoceros* has been used as a potential algae to harvest lipids for biofuels. It shows seasonal fluctuations in its abundance during different seasons in Cochin area. Studies made on their abundance showed that it was dominant during January-February in Cochin areas than premonsoon. *C. calcitrans* is dominant during post monsoon showed their tolerance and growth in highly saline condition.

Of the various sources of Arsenic in the environment, water born Arsenic probably possess the greatest threat to human health. The Arsenic concentration in natural waters ranges from 0.5-5,000 mg/L (Smedley and Kinniburgh, 2002).

Arsenic is known for its toxicity to humans and affects mainly the liver, kidneys, blood, bones, teeth and intestinal walls. The toxic action of Arsenic is by attacking-SH groups of an enzyme, thereby inhibiting enzymatic action. The enzyme producing cellular energy in the Kreb's cycle gets adversely affected. Toxic effects of Arsenic have already been reported in human beings (Borgono *et al*., 1997; Chakraborti *et al*., 1998). A high exposure to inorganic Arsenic can cause infertility and miscarriage in women, and it can cause skin disorders, low resistance to infection, heart disruptions and brain damage in both men and women (Chakraborti *et al*., 1998). A lethal dose of Arsenic oxide is generally regarded as 100 mg. Organic Arsenic can cause either cancer, or DNA damage. Exposure to high doses may cause certain effects to human health, such as nerve injury and stomachaches (Chatterjee *et al*., 1995).

The algae are the primary link in the aquatic food chain and any bio concentration in them ripple the effect on subsequent members of food chains or trophic levels. Since many water bodies are used for effluent discharge, some of which may contain the toxic heavy metals the present study was undertaken to study the effect of arsenic on the growth and physiology of *C. calcitrans*. The algae used for this study are native to marine ecosystem and were subjected to the heavy metal Arsenic usually present in polluted water bodies.

Material and methods

Standardization of the media

The suitable media for the better growth of the test species was determined by culturing the algae in different media such as Walne's media, Michel's media and Chu-10 media. From this it was concluded that, Walne's media was best for the optimum growth of the algae. *C. calcitrans* was cultured in different salinities such as 20 ppt, 30 ppt and 35 ppt. The most suitable salinity was found to be 35 ppt.

Seasoning of the sea water

From the preliminary studies on the seasonal abundance of algae, it was observed that *C. calcitrans* was found abundant during post monsoon period when the salinity was around 35 ppt. The sea water was collected during premonsoon and was kept for two weeks for seasoning under aeration for dechlorination.

The medium was prepared by dissolving necessary micro and macro nutrients in 1 litre sea water. The medium was autoclaved at 121° C for 20 minutes. After autoclaving 1 ml of trace metals, phosphate and silicate were added to it. Arsenic stock solution was prepared by dissolving 4.03 g sodium arsenate in 1 litre distilled water.

Inoculation of algae

The selected concentrations of Arsenic [0.01 ppm, 0.1 ppm, 1 ppm, 2 ppm, 5 ppm, 10 ppm, 20 ppm, and 40 ppm] were added to the conical flasks containing 500 ml medium. About 82 cells per 0.1 ml of *C. calcitrans* was used as an inoculum. For uniformity of the experiment, a definite volume (10 ml) of the inoculum was inoculated to each flask using a sterile pipette. A control was maintained for comparative assessment. The inoculum was well mixed in the medium. These were incubated under 14 hours light and 10 hours dark cycle. The light necessary for the growth was provided by using fluorescent tubes having 1200 Lux for 12 to 14 hours a day. The temperature of the culture media was maintained between 28 and 32°C.

Bioassay studies using C. calcitrans

The growth kinetics of the test species *C. calcitrans* was studied by counting the number of cells using Haemocytometer. The growth phase was found to be 16 days, after which there was a short stationary phase.Growth parameters selected for study was biomass (number of cells/0.1 ml.), productivity (mg/ml.), pigment content and the end products of photosynthesis -mProtein and Proline. Biomass determination was done from 2nd day onwards at every alternative day. It was determined on the basis of number of cells counted with the help of Haemocytometer and was expressed as the number of cells /0.1 ml. Productivity was determined from fourth day onwards.

The amount of Chlorophyll a, Chlorophyll c and Carotenoid were estimated by the method prescribed by Sadasivam and Manickam (1996). The pigment concentrations were expressed as mg/l. The amount of Chlorophyll a, Chlorophyll c and Carotenoid were calculated using the Larzsen and Parkin's equation.

Protein was estimated by Lowry's method at the end of the exponential growth phase. The amount of proline present in different samples were estimated at the end of exponential growth phase using the method prescribed by Sadasivam and Manickam (1996).

Results

This study was carried out to determine the effect of Arsenic on the growth and physiology of the Diatom *C. calcitrans* isolated from Cochin backwaters and the rate of removal of Arsenic using this algae.

There was gradual increase in biomass at lower concentrations such as 0.01 ppm, 0.1 ppm, 1 ppm, 2 ppm and 5 ppm (Fig. 1). In all these concentrations, the biomass was greater than the control. The maximum biomass was shown in 5 ppm arsenic concentration. More than two times increase in biomass was noticed in 5 ppm compared with the control. But a gradual decrease in biomass was observed above 5 ppm.

The rate of productivity was also similar to the biomass. In this case also, the productivity was more than the control in the concentrations of 0.01 ppm, 0.1 ppm, 1 ppm, 2 ppm and 5 ppm. Above these concentrations, there was decrease in productivity. The highest productivity was observed in 5 ppm (Fig. 2).

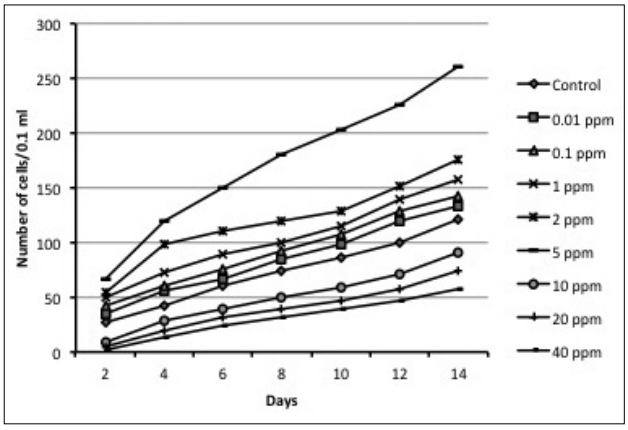

Fig 1. Effect of arsenic on the biomass of *Chaetoceros calcitrans*

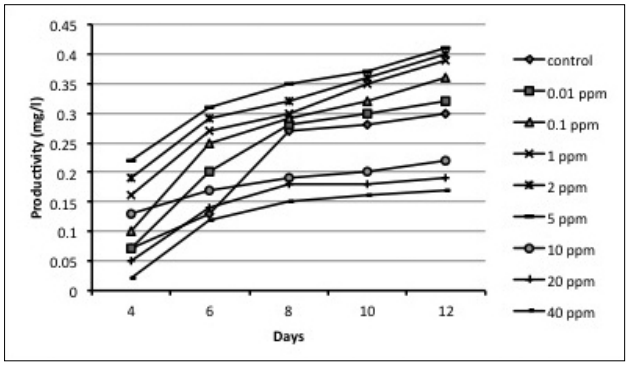

Fig. 2. Effect of arsenic on the productivity of *Chaetoceros calcitrans*

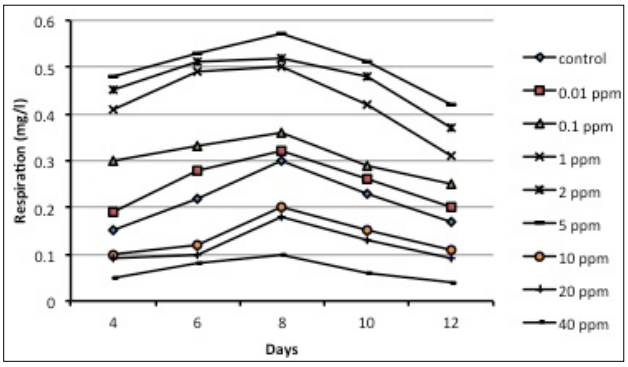

Fig. 3. Effect of arsenic on the respiration of *Chaetoceros calcitrans*

In the case of respiration, initially Arsenic was found to have stimulatory effect on respiration of the algae. But gradual decrease was noticed towards the end of the growth phase (Fig. 3). Maximum rate of respiration was noticed in 5 ppm. Above 5 ppm, there was decrease in the respiration. In high concentrations there was gradual decrease in growth. From this observation, it was found that the productivity and respiration were directly proportional to biomass.

The Chlorophyll content (both Chlorophyll a and Chlorophyll c) showed increase in lower concentrations up to 5 ppm (Fig. 4) where it was maximum. But in the higher concentrations (10 ppm, 20 ppm and 40 ppm) the Chlorophyll content was found to decrease. When compared to the amount of chlorophyll, the carotenoid was more in all concentrations. An increase in carotenoid level was found in all concentrations. The amount of carotenoid was more in higher concentrations of Arsenic.

The amount of proteins in lower concentrations showed gradual increase up to 5 ppm. About 45% increase in protein was noticed in 5 ppm. But above 5 ppm, like biomass, productivity and respiration, the amount of protein was found to decrease. A marked decrease in protein was noticed in 40 ppm when compared with the control (Fig. 5).

The amount of carotenoid and proline in high concentrations, even at 40 ppm, were more than the control (Fig. 6). The proline was found to be 8 times more than the control at 5 ppm, whereas it was found to be 3 times that of control at 40 ppm. So maximum removal of Arsenic was observed at 5 ppm.

Pronounced morphological changes were noticed at high concentration. The diatom was found to be settling on the lower surface of the flask and was seen as an entangled mass. This may be due to the mucilaginous secretion produced by the diatoms to protect itself from the toxic effect. Extra cellular mucilage protects the diatom cell from Arsenate.

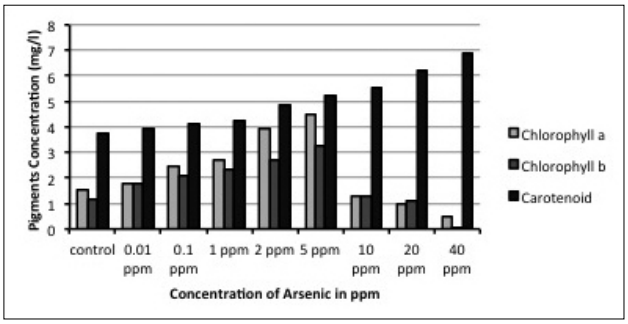

Fig. 4. Estimation of chlorophyll '*a*', chlorophyll '*c*' and carotenoid in different concentrations of arsenic

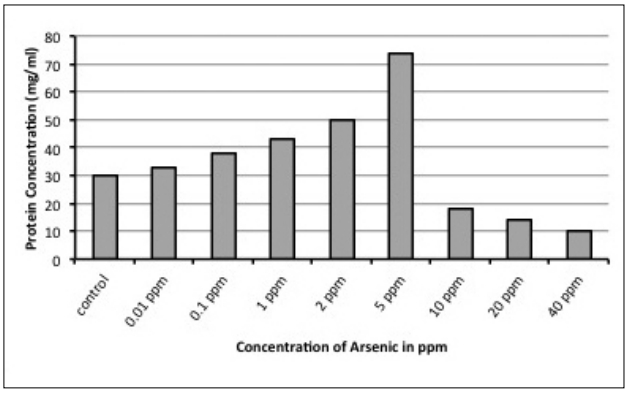

Fig.5. Protien content of *Chaetoceros calcitrans* in different concentrations of arsenic

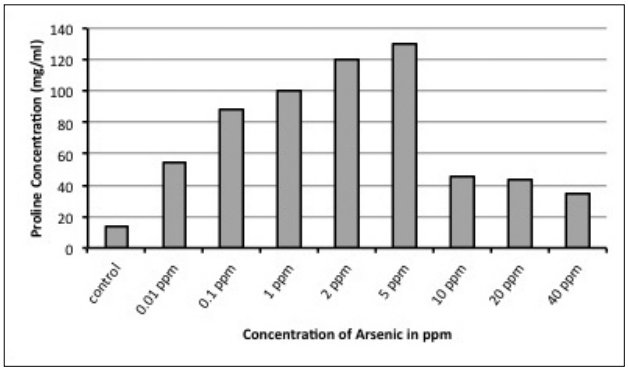

Fig. 6. Proline content of *Chaetoceros calcitrans* in different concentrations of arsenic

From this experiment, it was observed that the presence of Arsenic at lower concentrations with high salinity in the culture medium increases the growth of the algae. Arsenic was found to be stimulating growth of algae at lower concentrations such as 0.01 ppm, 0.1 ppm, 1 ppm, 2 ppm and 5 ppm. But algae show ease in growth rate with increase of Arsenic concentration above 5 ppm. In lower concentrations, up to 5ppm, the growth was more than the control whereas from 10 ppm onwards there was a decline in growth.

Discussion

Algae are the primary link in the aquatic food chain. Arsenic pollution is a severe issue in some of the aquatic environments. It is important to study the effects of Arsenic on the algal growth in order to assess the ecological risk of Arsenic pollution.

The bioassay studies were conducted to understand the heavy metal uptake from metal enriched solution. The effect of metal on the biomass/growth rate and the mechanism of metal tolerance were studied, thereby suggesting the importance of this algae in bioremediation and environmental cleanup operations.

Attempts have been made to correlate the concentration of metals in Cyanobacterium (Meenakshi *et al.*, 2004), in fresh water fishes (Shobha Rani *et al.*, 2000) and in selected microalgae (Samal *et al.*, 2004).

In the case of estuarine forms, salinity plays an important role in the metal uptake and growth. High salinity stimulates the metal uptake in *C. calcitrans*. In Cochin backwaters, during post monsoon season, salinity ranges from 26 to 35 ppt. During monsoon salinity remains low because of the heavy influx of fresh water from the nearby rivers and from the rainfall.

In the present investigation, it was found that the biomass increased at lower concentrations upto 5 ppm where as at higher concentrations it decreased. Similar change in the growth kinetics due to the effect of heavy metals on *Chlorella* was also reported by Sini and Manju (2014). The phenomenon of increase in biomass with sub lethal concentrations of metal was observed by Sharma (1987) in *Spirulina* species.

The productivity of *Spirulina platensis* got reduced significantly with higher concentrations of trace metals. (Aji and Kurup, 2010). In the present investigation, the rate of productivity and respiration was related to the biomass. Rate of productivity and respiration were maximum at 5 ppm concentration of arsenic and from their onwards rate of productivity and respiration decreased as evident from Fig. 2 and Fig. 3.

In the case of pigments, the gradual increase upto 5 ppm was noticed and with higher concentrations from 5 ppm onwards, the chlorophyll content was found to decrease as depicted in Fig. 4. The decrease in chlorophyll pigment may be due to the displacement of Mg from the chlorophyll molecules by Arsenic, resulting in changes in the fundamental characteristics as suggested by Saraiva, 1976. But the trend was different in the case of Carotenoids. In the case of Carotenoid, marked decrease was not noticed. In higher concentrations also, the carotenoid content was found to be high. It increased with increase in concentration of arsenic. Chintamani and Mohanti

(1988) found that Zinc grown cells contained less chlorophyll and it decreased with increasing Zinc concentration. The uptake of more Zinc might have caused certain enzymatic changes in the metabolic process, which caused yellowing even with growth promoting concentration (50 mg/ml) as time passed. The reduction in growth rate with increasing metal concentration was largely due to a gradual change in chlorophyll a concentration. The reduction in chlorophyll was either due to an inhibition of chlorophyll biosynthesis or due to a breakdown of pigments on their precursors (Sen and Bhattacharya, 1993).

Barambhatt and Rinku (2012) reported that the accumulation of Cr ion increased proteins content from 2 ppm to 10 ppm, but after 10 ppm it was decreased day by day in *Pithophora* species. In the present study, accumulation of Arsenic increased the protein content from 0.01 ppm to 5 ppm, but after 5 ppm there was gradual decrease in subsequent days (Fig. 5).

Even though a sudden increase in biomass observed due to initial metal uptake, slowly toxic effect was noticed in the metabolic activity of the cell. This was reflected in the end products of photosynthesis and protein. It was reported that Arsenic is known to inhibit the synthesis of insoluble basic compounds including protein in sensitive cells, not in tolerant cells (Meenakshi *et al.*, 2004). Arsenic exerts its toxic action by attacking the SH - group of the enzyme. By virtue of its chemical similarities to Phosphorous, it interferes with some biochemical processes involving Phosphorous. This was reflected in the decrease in the end product, the protein. Arsenic compound at high concentration coagulates proteins possibly by attacking the sulphur bond containing the secondary and tertiary structures of protein.

Proline has been shown to play an important role in ameliorating environmental stress in plants and microorganisms including heavy metal stress (Surasak *et al.*, 2002). Enany and Issay (2001) investigated the accumulation of proline in *Scenedesmus armatus* and correlated it with protein content. The amount of proline in the present investigation was more than the control in all concentrations. The *C. calcitrans* in 5 ppm concentration produced high amount of proline (Fig. 6) which indicates the environmental stress. Stress due to the heavy metal toxicity stimulates the algae to produce high amount of proline, to overcome the stress.

Since the test species *C. calcitrans* was isolated from the Cochin backwaters, the tolerance limit to metal pollutants will be more when compared to fresh water forms. So this diatom can be used for the removal of Arsenic from Arsenic polluted waters at low concentration, especially paddy fields and in aquatic environment where pesticide pollution is found to be high. By suggesting the threshold level of toxicity of this metal, it will be easy for environmentalists and law makers to suggest a tolerance level, beyond which it is toxic. The speedy growth rate and the potential to produce a high biomass within the stipulated time, is an important aspect that can be considered for its usage in phytoremediation studies. The bioassay was carried in vitro. But as a pilot study it can be used as a specific application of environmental biotechnology for the management of Arsenic pollution and keeping in view of the concept of sustainable development.

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