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EFFECT OF ARSENIC TOXICITY UNDER DIFFERENT NITROGEN REGIME ON NITRATE UPTAKE IN CHLORELLA VULGARIS

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ABSTRACT

This study examined the effect of 25.0 and 50.0 µM arsenic (As) on nitrate uptake in Chlorella vulgaris when grown under different nitrogen regime. The changes in nitrate uptake of test alga exposed to As under different N regimes show that the control cultures of test alga showed maximum nitrate uptake in N enriched cells. The nitrate uptake under normal N level was decreased by 18.05% (25.0 µM As) and 26.24% (50.0 µM As). Likewise, under N deficient condition, both concentrations (25.0 and 50.0 µM) of As reduced the nitrate uptake of the test organism by 78.14% and 88.46%, respectively. The effect of As was same during excess N condition, where nitrate uptake of the test organism was reduced by 8.75% and 20.52% at 25.0 and 50.0 µM As, respectively. More experimental and molecular evidences will further enhance our understanding about the relation of nitrogen regimes with metal tolerance in algae.

1. INTRODUCTION

Arsenic (As), a toxic metalloid, is known to adversely affect all the biota and thereby create severe threat to the environment. As and its compounds have the tendency to accumulate and persist in the cells consequently impose adverse effect on ecosystem [1].

Parent rocks, volcanic eruption, industrial and household waste discharge, and fuel combustion are the major sources of As release in the environment [2, 3, 4]. Toxicity of As cause protein and enzyme oxidation, lipid peroxidation, DNA oxidation and further generates reactive oxygen species (ROS) like, superoxide radical, hydroxyl radical, singlet oxygen etc [5, 6].

Algae are the important primary producers and prevalent inhabitant of aquatic systems. Therefore, these tiny organisms first noticed and encountered any change due to the discharge of chemicals, including As, in the aquatic environment is by. It has been demonstrated that the higher concentrations of As in water sharply reduce the cell viability of algae [7]. Moreover, As interferes with pentose phosphate pathway which leads to impairment in photosynthesis of algae [8, 9].

Phormidium laminosum and *Scenedesmus acutus* when exposed to different concentrations of As showed a significant reduction in protein and carbohydrate content [10, 11]. Several environmental factors, including the nutrient status of the aquatic habitat, affect the algal growth. Among these nutrients, nitrogen and phosphorous are two key elements and are major constituent of proteins, nucleic acids, pigments, enzymes and various metabolic processes by which algal cells use solar energy to produce their food through photosynthesis [12]. Since nitrogen is an important constituent of biomolecules, therefore, it plays a vital role in living cells [13]. Therefore, for the biosynthesis of amino acids and proteins the nitrogen metabolism, including the uptake and assimilation of nitrate, is essential for plants [14].

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Plants and algae have been able to utilize this inorganic nitrogen in form of nitrate and ammonium ion [15]. An adequate supply of nitrogen is necessary to ensure high production rates of algal biomass, however, the variation in nitrogen regime can influence the growth and general metabolism of algal mass culture [16, 17].

Chlorella vulgaris is a unicellular, mix trophic, auto spore forming green algae found in fresh water bodies [18]. The biochemical and physiological properties of *Chlorella vulgaris* is very much alike with higher plants. Additionally, its photosynthetic apparatus is also similar to higher plants. However, the growth of *Chlorella vulgaris* is very fast in comparison to plants. Therefore, algae may serve as a useful biomarker of arsenic toxicity in water ecosystem [19].

Unfortunately, the interaction of nitrogen with As toxicity is poorly studied. Additionally, not much information is available about the interaction of nitrogen with As-induced changes in algae. Therefore, the present study was conducted in *Chlorella vulgaris* to evaluate the interaction of nitrogen with As mediated responses on nitrate uptake.

2. MATERIAL AND METHODS

2.1. Test organism, culture conditions and experimental design

A fast growing freshwater microalga *Chlorella vulgaris* L. (chlorophyta) obtained from the laboratory of Professor JP Gaur (Banaras Hindu University) was used as the test organism due to its fast growth and easy handling in physiological studies. The test alga was cultivated in 4 times diluted BG-11 culture medium [20].

The cultures were incubated in an air-conditioned culture room at standard conditions of light (72 $\mu mol.m^{-2}.s^{-1}$ PAR) and temperature (24 ± 1 °C) with 12:12 h light and dark cycle. The pH of the culture medium was adjusted to 6.8 and cultures were shaken manually 2-3 times daily.

The exponentially growing culture of *Chlorella vulgaris* was cultivated in nitrogen free medium for 7 days. Cells were harvested and transferred to 250 ml Erlenmeyer flasks each containing sterilized culture medium with three different levels of nitrogen, e.g., deficient (0.5 gL^{-1}), normal (1.5 gL^{-1}) and excess (2.5 gL^{-1}). These concentrations of N were selected based on their effect on the growth of the test alga. The initial cell density of the suspension was 10^4 cells ml^{-1}.

After 2 days of acclimatization, freshly prepared sterilized stock solutions of $NaH_2AsO_4.2H_2O$ was added to the culture flasks to obtain the 25.0 and 50.0 µM concentrations of As in the medium. These concentrations were selected after screening their effects on the growth of the test organism and were able to inhibit the specific growth rate of the test alga by approximately 25% and 50%, respectively.

The culture medium without enrichment of As was considered as control and all flasks were incubated for 7 days. Measurements were made in triplicate to study the effect of As treatment on nitrate uptake by test organism under different nitrogen regime.

2.2. Measurement of nitrate uptake

The nitrate uptake was measured by using brucine sulfuric acid method as per Nicholas and Nason [21]. A known volume of algal suspension was centrifuged and to the supernatant (1.5 *ml*), 0.6 *ml* brucine solution was added. A 1.5 *ml* concentrated H_2SO_4 was added to this solution. The mixture was heated at 100 ºC for 10 minutes.

The absorbance of the resulting yellow color was measured at 410 nm on *UV-VIS* spectrophotometer. Thereafter, nitrate uptake was calculated as described by Tripathi et al. [22, 23].

3. RESULT AND DISCUSSION

The changes in nitrate uptake of test alga at 25.0 and 50.0 μM, as under different *N* regimes are shown in Figure 1.

It is evident from this figure that in N enriched cells the control culture of test alga showed maximum nitrate uptake. Moreover, the nitrate uptake under normal *N* level was decreased by 18.05% and 26.24% when *Chlorella* cells were treated with 25.0 and 50.0 μM As, respectively.

Similarly, under *N* deficient condition, both concentrations (25.0 and 50.0 μM) of As significantly ($P < 0.05$) reduced the nitrate uptake of the test organism by 78.14% and 88.46%, respectively. The effect of As was same during excess *N* condition, where nitrate uptake of the test organism was reduced by 8.75% and 20.52% at 25.0 and 50.0 μM As, respectively.

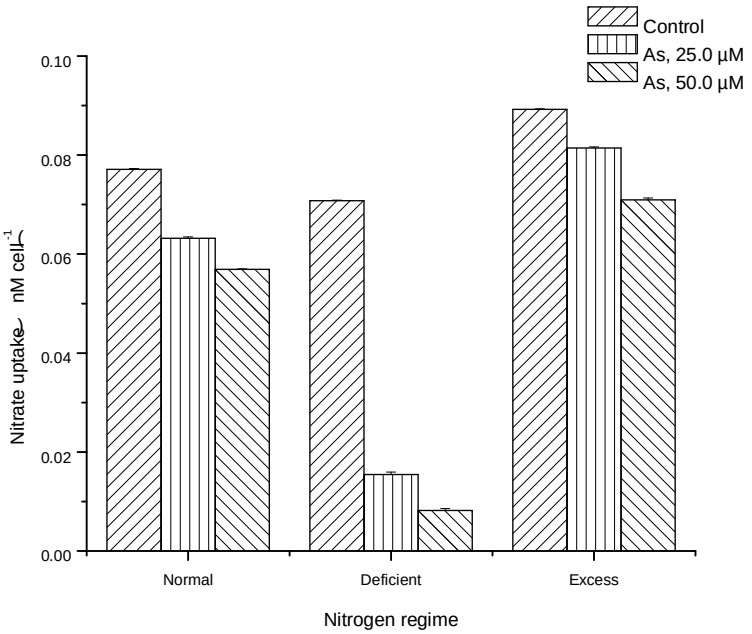

Figure 1 Nitrate uptake in *Chlorella vulgaris* under different nitrogen regimes.

The maximum nitrate uptake in *Chlorella vulgaris* without As exposure was observed under high N regime which is consistent with the findings of Pedersen et al. [24] and Wu et al. [25]. Both studies reported that the nitrate uptake in *Porphyra* sp. and *Fraxinus mandshurica* seedlings were significantly increased when treated with high N concentrations. Contrary to this the findings of Shi et al. [26] suggests that the cells of *Alexandrium tamarense* grown in low N cultures had a higher nitrate uptake rate. The nitrate uptake in *Chlorella vulgaris* was reduced when treated with As concentration under different N regimes (Fig. 1). The maximum reduction was observed under N deficient condition. It is well known that nitrogen is generally taken up by algae in the form of nitrate through an active transport system which is an ATP dependent process [27]. In nitrogen starved cells which already suffer with low N supply, As toxicity causes a reduction in ATP pool and thereby reduce the ability of the test alga to sequester nitrate from the surrounding medium [28].

As a concluding remark, more experimental and molecular evidences are required to further understand the relation of nitrogen regimes with metal tolerance in algae.

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