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ORIGINAL ARTICLE

Effect of Lead on Chlorophyll Accumulation in *Asterarcys quadricellulare*

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ABSTRACT
Algae will grow majorly in aquatic environments which are the best sources of food, medicine, energy etc. Algae-based bioproducts are popular due to its easily available in nature and are efficient photosynthetic organisms. Therefore, the enhancement of algal biomass is a one the recent issues in recent times. Apart from various key components for growth and development of algae, the heavy metals also play a crucial role. Lead is one of the most important environment pollutants which deserves the increasing attention in recent decades. In the current work, fresh water microalga, Asterarcys quadricellulare was used to screen with the lead metal. Various concentrations of lead (0.0, 0.5, 1.0, 2.0, 4.0 and 6.0 mg/L) were used along with algal tris-acetate phosphate medium. TAP with 2.0 mg/L lead dose showed the improvement of biomass compared to untreated to control. Current work may be useful for biomass production as well heavy metal remediation research using algal species.
Keywords: *Asterarcys quadricellulare, Heavy metal, Lead, Chlorophyll, Biomass.*

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INTRODUCTION

Algae belong to primitive group of photosynthetic organisms that prevail in most of the ecosystems. Algal species have the capability for producing several beneficial products due to their efficient photosynthetic capacity, short life span and easy to handle under *in vitro* conditions [1]. They are classified into terrestrial and aquatic species based on habitat with unicellular and multicellular nature. Over the last few decades majority of the researchers are focusing on algae, diatoms, bacteria etc., for generation of bioproducts [2]. Specifically, production of biofuels from algal feedstock has been interesting research [3, 4]. In addition, few works on biosorption studies were observed using various algal species [5]. Hence, it is important to improve the biomass to get bioproducts including biofuels.

Due to population explosion, utilization of biomass by the human society is increasing regularly since olden days. Biomass is developed from organic materials which is generally renewable and sustainable source in various forms [6]. Biomass can be used majorly for food, medicine and fuel preparation depends on the biological source. Each and every region has its own locally available biomass feedstocks through agriculture, forest and other aquatic sources [7]. Some of the most common biomass feedstocks are agricultural residues, food waste, forestry materials, animal byproducts, energy crops and urban wastes [8]. Algae can drive photosynthetic reduction of carbon dioxide into carbohydrates and hydrocarbons and their subsequent conversion into bioproducts [9]. These stocks can be made into liquid fuels, thermal, electric power and other products. Particularly, both organic and inorganic elements play a major role in algal metabolism apart from vitamins and growth hormones [4]. Specifically, certain elements are involved in biochemical pathways of algae which decide the content of biomass.

Generally, heavy metals are classified into essential and non-essential which dictates the content of biomass in plants including algae [10]. Figure 1 illustrates the sources of heavy metals from both natural and anthropogenic activities. Apart from toxic non-essential elements, accumulation of excess of essential elements causes the production of reactive oxygen species (ROS). Lead (Pb) is one among the metallic toxic pollutants originating from industrial wastes, combustion of fossil fuels by vehicles and use of agrochemicals [11]. Lead may available be in the atmosphere as dust, mist, smoke and in soil as a mineral. Lead normally used for preparation of batteries, paints, PVC plastics, pesticides and also used as fuel additives [12]. Though it is available in the small amounts in the system, its capability of sustenance and ability to perform biochemical functions is extraordinary which is noxious most of the occasions. Specifically, lead enters into the food chain and can subsequently endanger human and animal health. Under normal conditions, lead inhibits certain enzyme activities in many biochemical pathways.

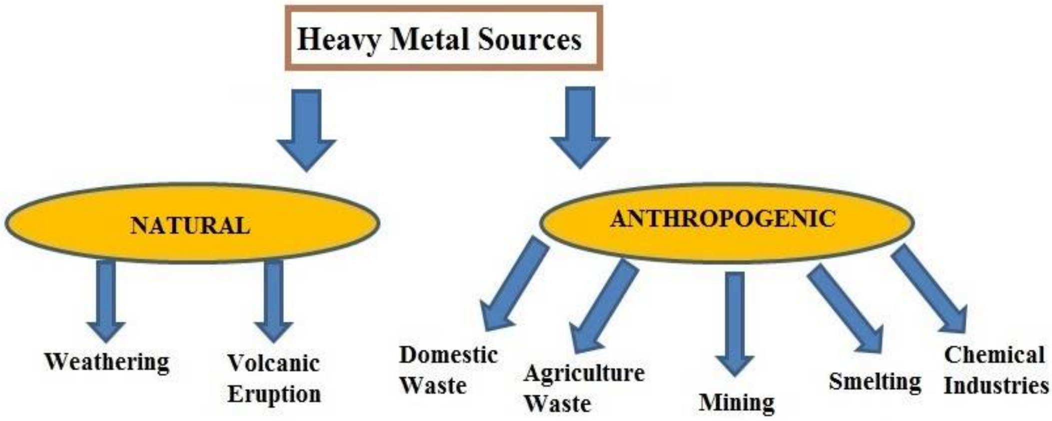

Figure 1. Various sources of different heavy metals

In the present investigation, a green alga namely *Asterarcys quadricellulare* was used for the experiments to augment the biomass production by altering the various concentrations of lead nitrate in the TAP medium under *in vitro* conditions. *Asterarcys quadricellulare* belongs to freshwater algae with unicellular in nature [13]. To the best of our knowledge, for the first time, screening was carried out to using lead in this alga to know its effects on chlorophyll accumulation.

MATERIAL AND METHODS

We selected the *Asterarcys quadricellulare* as a source of freshwater green algal material from University of Madras, India to carry out the present work. Collected algal cells were *in vitro* cultured at 25°C in TAP (Tris-acetate-phosphate) media by maintaining pH 7.0 (Elico limited, India) and stored as glycerol stock and kept at -80°C for further usage as per the procedure of Paramesh and Chandrasekhar [14]. All the glassware (Borosil, India) and vessels were cleaned properly and finally oven (Kemi, K04.3, Ernakulam, India) dried to perform further experiments.

Table 1. Algal media with various lead nitrate doses

S. No	Heavy metal	Heavy metal concentration
1	Lead nitrate	TAP with 0.0, 0.5, 1.0, 2.0, 4.0 and 6.0 mg/L

Preparation and sterilization of media

TAP medium used in the current work contains Tris base, TAP salts, phosphate solutions, trace elements and glacial acetic acid. In addition to TAP medium, we added lead nitrate using 0.0, 0.5, 1.0, 2.0, 4.0 and 6.0 mg/L concentrations (Table-1). After adjusting the pH 7.0, all the media made to a known volume in conical flasks for *in vitro* cultures [9]. Media with various doses of heavy metal were autoclaved for 15 min at 15lbs/in^2 in an autoclave (Valve, RBI, Italy). After the completion of sterilization, all the algal media were removed from the autoclave and the flasks were placed in LAF (laminar air flow) chamber (Hitech products, Bangalore, India).

Inoculation and maintenance of culture conditions

Prior to inoculation of *Asterarcys quadricellulare*, the LAF chamber was cleaned with ethanol. All the inoculation requirements i.e., algal tubes, sterilized loops, lamp etc., were transferred inside the laminar flow chamber. Inoculation was carried out with clean hands (applied with seventy percent ethanol) using sterilized loops and also using micropipette. Inoculated flasks were maintained in a culture room at 25°C and 24 h photoperiod using white fluorescent tubes.

Chlorophyll estimation

Chlorophyll estimation was carried using modified methods of Arnon [15] and Varaprasad et al. [16] with a spectrophotometer (Shimadzu UV-1800). Collect 100µL each *A. quadricellulare* culture as aliquot solution in to 1ml vials which already having 900µl of 80% acetone and mix well, later centrifuge at 5000 rpm up to 5 min. Further, collect the supernatant solution and then take O.D values at 663 nm (Chl-a) and 645nm (Chl-b) for chlorophyll contents and later total chlorophyll content were estimated using the standard procedure. A minimum of three replicates were involved in each set of experiment and mean values were used for statistical analysis.

RESULTS AND DISCUSSION

At present, fresh water green alga *Asterarcys quadricellulare* was used to perform heavy metal screening in the TAP medium. TAP medium alone used as a control and TAP along with various doses of lead nitrate were used as experimental treatments. Biomass analysis was done by visual observation of culture growth day by day and further the chlorophyll estimation was carried out using spectrophotometric method. The results of the current experiments were documented below (Figs. 2 and 3).

Total chlorophyll estimation in *A. quadricellulare* cultures

In *A. quadricellulare*, we observed the visual growth started around 12 hrs. TAP along with lead nitrate was exhibited best growth at 2.0 mg/L when compared to untreated control (Figs. 2 and 3). Hee et al. [11] observed the lead impacts on *Chlorella vulgaris* in turn physico-chemical properties of water. Further, the cultures were used for estimation of chlorophyll content in TAP alone, TAP along with lead nitrate media. The total chlorophyll content was augmented in TAP with 2.0 mg/L lead nitrate medium when compared to control i.e., TAP alone medium (Fig. 3). This is almost 2.5-fold more total chlorophyll contents when compared to control cultures. In contrast, higher concentration of lead reduced the total chlorophyll contents. Similarly, De Schamphelaere et al. [12] observed the toxicity of lead in micro algal species growth and development. Also, Dao and Beardall [17] observed the production of reactive oxygen species in algal species when exposed to lead metal.

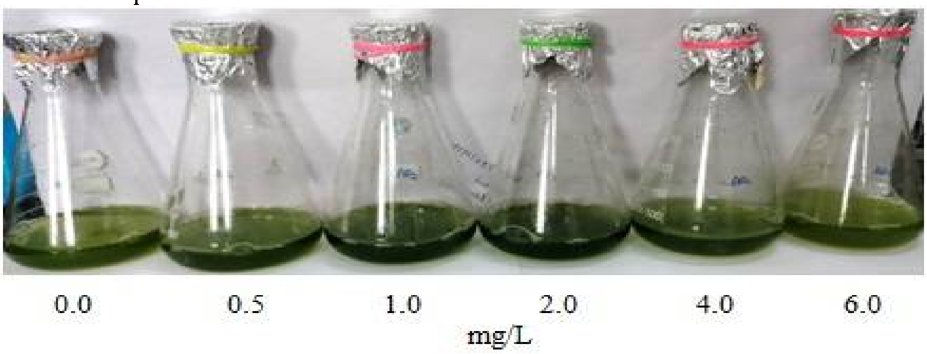

Figure 2. Algal growth in lead nitrate treatment

Moreover, 0.5 and 1.0 mg/L of lead nitrate cultures generated more total chlorophyll contents when compared to control (Figs. 2 and 3). But chlorophyll contents were reduced in TAP with 4.0 and 6.0 mg/L media (Fig. 3). In conclusion, TAP with lead metal also proved better for biomass enhancement in algal cultures.

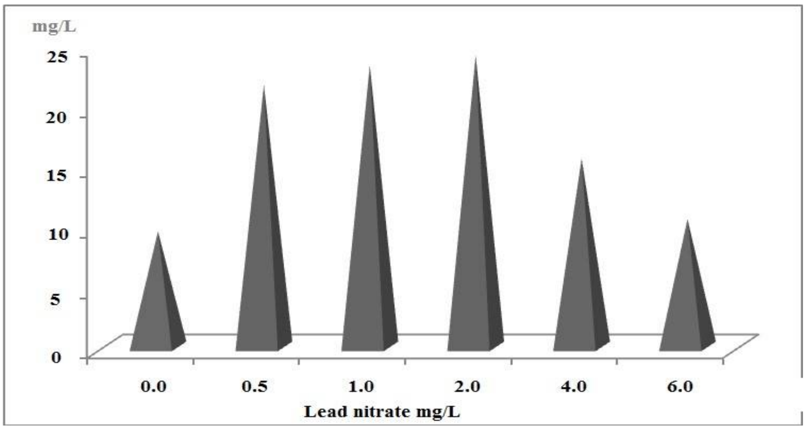

Figure 3. Total chlorophyll contents in lead treated algal species

CONCLUSIONS

In the present investigation, *A. quadricellulare* showed different growth pattern in various lead concentrations. TAP along with 2.0 mg/L cultures are exhibiting enhanced biomass compared to TAP medium alone. In contrast, higher concentration of lead inhibiting the growth as well chlorophyll accumulation. Overall, present work may be helpful in future algal biomass research in turn for generation of bioproducts. Also, this work may useful for biomass-based renewable energy research area.

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CONFLICT OF INTERESTS

Authors declared that there is no conflict of interest for this study.

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