Monitoring of the Interaction of Chromium (VI) on the Growth Profile of Chlorophyceae Marine Microalga *Chlorella vulgaris* in Controlled Laboratory Condition

Sucheta Sadhu[1*] and Kirubagaran R.[2]

***Abstract*—**Chromium (Cr) is ubiquitously present in the aquatic environment. From various natural and anthropogenic sources Cr (VI) reaches to the aquatic ecosystem through weathering and surface runoff. The nature of metals is conservative which leads to the build-up of metal concentration in aquatic system. Microscopic phytoplankton imbibes these metal ions as nutrient irrespective of their essentiality and eventually incorporates to various *in situ* assimilatory processes. In this study, we report the interactive effect of Cr (VI) on the Chlorophyceae marine microalga *Chlorella vulgaris* (NIOT-74, NCBI Accession No: JF894250.1) during the exponential growth phase of the cells on its growth profile and bioconcentration factor in laboratory condition.

***Keywords*—** BCF, *Chlorella vulgaris*, growth profile, Cr (VI).

I. Introduction

CHROMIUM (VI) is ubiquitously present in the aquatic environment. Due to the non degradable nature of metal, it keeps accumulating in the environment after mobilizing through run-offs metallurgical wastewater (June *et al.*, 2010) from source to the sink. As a result of this, the plethora of metal xenobiotics builds-up in the aquatic ecosystem. To maintain the homeostasis of the aquatic ecosystem and its surroundings, the total ion pool is regulated in terms of its mass balance. Monitoring of the environmental and physiochemical parameters of the aquatic system reflects some insight about the aquatic environment but it fails to forecast about the growth profile of the microalgae. Microalgae form the base component of the food chain of the natural environment and are invariably affected by insidious metal ions. Microscopic phytoplankton imbibes these metal ions as nutrient irrespective of their essentiality and eventually participates to various *in situ* assimilatory processes. There are numerous studies available on the interaction of metal and microalgae. The quantitative studies on metal ions are scanty. Recently, many technologies have been opted to mitigate the metal pollutions in the aquatic environment. Literature reveals chemical treatment, use of adsorbent, incineration; land fill, biological treatment etc. depending on the pollutant criteria these methods are suitable but the concern on secondary pollutant generation still remains in debatable condition. Biological mitigation processes have been proven as green technology to detoxify metal xenobiotics or toxicants as the toxicants gets converted into the nontoxic entity of the corresponding metal through *in situ* cellular mechanism. The challenges pops-up over here is how best this cellular efficiency can be utilized in terms of metal specificity, in terms of metal concentration and furthermore the biotic and abiotic factors involved during the process. Though biological mitigation goes hand in hand with green technology, yet it is not devoid of challenges in this in view of secondary pollutant generation. Recent studies indicate the detoxification property of microalgae (Jeewan *et al.*, 2014), however, yet there is a need to bridge the lacuna in terms pollutant specificity, suitable species selection, duration of exposure and aftermath fate of the species upon exposure into the particular metal xenobiotics. Use of biological arm to gun down the toxic xenobiotics seems towards a win-win situation.

In this study, we report the interactive effect of Cr (VI) on the Chlorophyceae marine microalga *Chlorella vulgaris* (NIOT-74, NCBI Accession No: JF894250.1) in triplicate against control over the exponential growth phase of the cells on its growth profile and bioconcentration factor in laboratory condition.

II. Materials And Methodology

A. The experimental method

The experiment was framed based on the Organization for the Economic Cooperation and Development (OECD 201) protocol.

B. Cultivation of Microalgae and Growth Media

The axenic monoculture of the marine microalga *Chlorella vulgaris* (NIOT-74, NCBI Accession No: JF894250.1) was received from NIOT's marine microalgal collection bank. The culture was grown in filtered seawater enriched with f/2 media. The cultures were acclimatized in experimental condition upto 5^{th} generation. The experiments were carried out exposing the cells in five dissimilar concentrations of Cr (VI) against control amid 54 µmol $m^{-2}s^{-1}$ photosynthetically active radiation (PAR) intended for 12:12 h (dark: light) photoperiod. The

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cultures were swirled gently thrice daily to prevent cell clumping.

C. Toxicant

Potassium dichromate ($K_2Cr_2O_7$), MERCK, was used to prepare Cr (VI) stock solution.

D. Treatment

An initial inoculam of the axenic monoculture of *Chlorella vulgaris* was inoculated in the Erlenmeyer flask (500 mL) which contained 200 mL of seawater enriched with f/2 media. The culture flasks consisting of five dissimilar concentrations of Cr (VI) ranging from 50 to 652 µg/mL were supplied in triplicate against control. The five concentrations of Cr (VI) were selected for the above experiment was on the basis of results of a range finding test conducted earlier.

E. Measurement of Growth Parameters

Algal cells, cultured for different durations at various range of concentrations were counted using haemocytometer by visualizing under a microscope (Karl Zeiss Axioscope2).

F. Determination of Dry Weight

Dry weight was measured following the protocol given by Zhu and Lee, 1997. Data were expressed as µg mL^{-1} algal suspension.

G. Metal Analysis

The uptake of Cr (VI) in cells was determined by ICP-OES (VARIAN 725-ES) after harvesting. Also the corresponding media and matrix were analyzed as prescribed by Grass hoff *et al.* with little modification. Alga was harvested at 96 h by centrifugation (4000g; ca.10000 rpm) for 15 min. All lyophilized algal biomass was weighed and then digested with concentrated ultra-pure HNO_3 and H_2O_2 (30%) (1:4) in pre cleaned, leaded, 100 mL Teflon vessels. After digestion, the samples were analyzed for metal content by ICP-OES. Blanks and spikes were analyzed to validate the digestion process of spectroscopic analysis, obtaining 95% recovery. In addition, 0.5 µg/mL of multi-elemental standard was analyzed upon every 10 samples to monitor the matrix effects on the analytes and for quality assurance and quality control.

H. Calculation of BCF

The BCF was calculated as defined by Brooks and Rumbsy (1965).

G. Statistics

Results were tested by one-way Analysis of Variance (ANOVA). ANOVA effects and treatments were considered significant when $p < 0.05$.

III. Results And Discussion

Microalgal phenotypic characteristics get impaired due to heavy metal exposure. The results during our investigation reveal that the duration and exposure concentration of heavy metals leads to retardation in growth profile of the exposed microalga. The alga was found to tolerate high concentration (652 µg mL^{-1}) of Cr (VI). The changes observed were found to be significant ($p < 0.05$) at all concentrations and durations as compared to control. All experimental values reported in triplicate and based on Mean ± S.D.

Our results show congruency with the results of Horcsik *et al.* (2006), June *et al.*, (2010) and Sucheta *et al.* (2016) too. Metals at lower concentrations is desired for the nutrition of microalgae, however, it is reported that chromium is not an essential (Sharma *et al.*, 2015) metal for microalgal nutrition or to perform cellular functions.

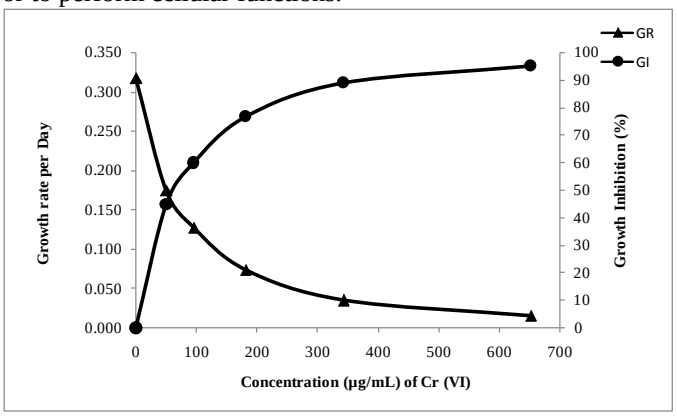

Fig1. Growth rate and growth inhibition profile of *C. vulgaris* during Cr (VI) exposure at 96 h.

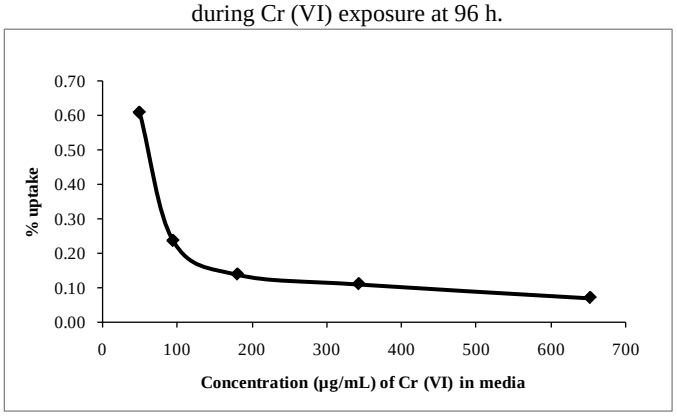

Fig2. Correlation of Cr (VI) uptake at different Cr (VI) exposure concentrations.

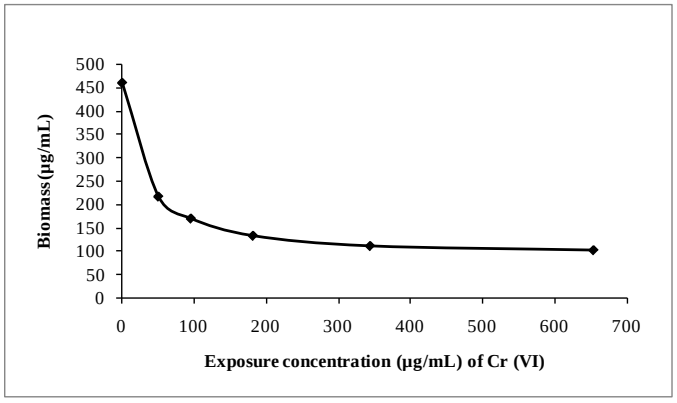

Fig3. Biomass profile at different Cr (VI) exposure

The growth retardation (Fig1.) of *C. vulgaris* can be attributed to the existence of the Cr (VI) itself. Cr (VI) accelerates to generate the free radical during *in situ* cellular assimilation process. Data analysis reveals that the % uptake of metal (Fig2.) is showing a declining trend with increasing exposure concentration.

Biomass is following a decline (Fig3.) trend as the exposure

concentration of Cr (VI) is in increasing order. At harvest, a prominent reduction (Fig4.) of Cr (VI) was observed indicating the bioremoval efficiency of *C. vulgaris*.

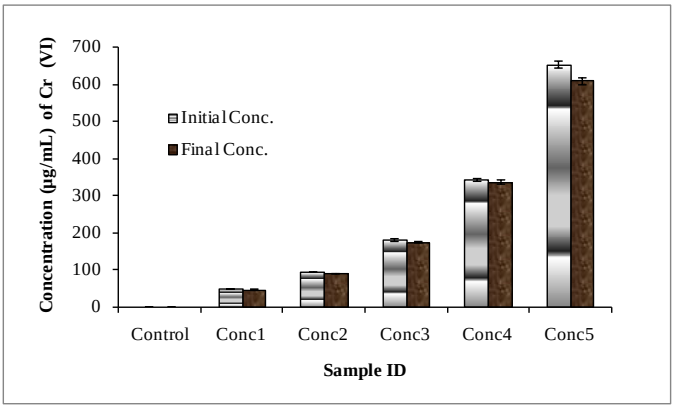

Fig4. Cr (VI) removal profile at 96 h during harvest.

IV. Conclusion

In conclusion, in this experiment it is found that the *C. vulgaris* can tolerate up to 652µg/mL of Cr (VI), however % accumulation is very less as the alga is exposed to higher concentration, though the removal efficiency increases with increasing exposure concentration of Cr (VI).

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