Environment Conservation Journal 16(3) 67-71, 2015
ISSN 0972-3099 (Print) 2278-5124 (Online)
Abstracted and Indexed

Impact of cobalt chloride on growth and biopigments of *Chlorella Vulgaris*

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Received:27.06.2015 **Revised: 12.09.2015** **Accepted:03.10.2015**

Abstract

***Chlorella vulgaris* is single cell green algae, which has many nutritive and pharmaceutical values. The effects of cobalt chloride on growth, chlorophyll a, chlorophyll b and carotenoids of *Chlorella vulgaris* were determined. The growth in terms of optical density, dry weight and fresh weight was slightly increased with increasing concentrations of $CoCl_2$ (upto 2ppm) but in higher concentrations (>2ppm) growth was reduced constantly, best growth was found in cultures treated with 2ppm cobalt chloride. The results of biopigment analysis were also correlates with growth results, highest amount of biopigments were found in moderate concentrations of $CoCl_2$. Results indicate the tolerance range of algae for heavy metal and useful to increase its nutritive value.**

OD: Optical density, FW: Fresh weight, DW: Dry weight, ppm: Parts per million, Chl: Chlorophyll, HM: Heavy metal

Keywords: Algae, Carotenoid, Chlorophyll, Heavy metal.

Introduction

Microalgal species are useful for production of biofuels and pharmaceuticles, food industry, waste water treatment and life support in space (Benemann et al. 1980; Borowitzka 1988; Glombitzka and Koch, 1989; Goldman 1979). Among these *Chlorella vulgaris* is an attractive food source because it is a good source of proteins and other nutrients. *Chlorella vulgaris* is a single - cell green algae, belonging to the phylum Chlorophyta. It is spherical in shape, about 2 to 10 μm in diameter, and is without flagella. Through photosynthesis, it multiplies rapidly, requiring only carbon dioxide, water, sunlight, and a small amount of minerals to reproduce. When dried, it is about 45% proteins, 20% fat, 20% carbohydrates, 5% fibers and 10% minerals and vitamins (Belasco 1997). Many pollutants like pesticides, oil hydrocarbons, heavy metals as well as thermal and radioactive pollution can get into aquatic environments after direct or indirect release from industries, agriculture and households (Fathi *et al*., 2008).). As an important group of these various chemical substances, heavy metals may be deposited into all ecosystems (Mutlak *et al*., 1979).The disturbance of aquatic ecosystems provoked by heavy metal pollution from industrial and domestic sources has as consequence the loss of biological diversity, as well as increased bioaccumulation and magnification of toxicants in the food chain (Pena-Castro *et al*., 2004). Cobalt is not only essential but also beneficial element. It is essential for many microorganisms including some microalgae. It forms a part of cobalamin, a component of several enzymes in nitrogen-fixing microorganisms, whether free living or in symbiosis (Kuyacak *et al.* 1989). Cobalt is distributed widely in algae, including microalgae: *Chlorella*, *Spirulina*, *Cytseira barbera*, and *Ascophyllum nodosum*. Alginates, such as fucoiden, in the cell wall play an important role in binding cobalt in the cell-wall structure (Ryrdina *et al* 1983).The aim of the present study is to evaluate the influence of cobalt on growth and biopigments of *Chlorella vulgaris.*

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Material and Methods

Isolation and purification of algae

The experimental organism *Chlorella vulgaris* was isolated from Mansagar lake (fresh water lake) and maintained at Department of Botany, University of Rajasthan in jaipur, Rajasthan (India). Single cell colonies were grown on solid media by serial dilution and subjected to repeated sub culturing in sterilized liquid nutrient media.

Nutrient media and culture technique

The cultures were grown and maintained in modified zarrouk's medium (Zarrouk 1966) at 26±2 ºC with a photoperiod of 16 hrs light and 8 hrs dark. The cultures were kept in 500 ml conical flasks and repeatedly subcultured in 15 days before treatment.

Treatment of cultures

The optical density at 670 nm was set to 0.1 for each flask. Each flask containing 250 ml cultures were treated with different concentrations (0.5ppm, 2ppm, 4ppm, 6ppm) of cobalt chloride. The treated cultures were analyzed for growth and their pigments after every 8^{th} day of culture.

Determination of growth and growth rate

Growth was followed through optical density, fresh weight and dry weight. Optical density was recorded with the help of photo colorimeter at 670 nm. For fresh weight and dry weight 50 ml of cultures was centrifuged every time. The palate was weighed for fresh weight and it was dried at 37 ºC in an oven for overnight for dry weight. Growth rate was calculated from dry weight by Guillard (1973) equation.

μ (divisions/day) = $3.322(\log DW_2 - \log DW_1)/t_2 - t_1$

Estimation of pigments

Spectrophotometeric methods were used for pigment estimation of deferent cultures of *Chlorella vulgaris*. Parson and Strickland method (1965) was used for estimation of chlorophyll content and Jensen (1978) method was used for carotenoid estimation.

Results and Discussion

Growth analysis (OD, FW, DW and growth rate) of $CoCl_2$ treated *Chlorella vulgaris* cultures shows different growth patterns (Figure 1-3), however all cultures started with similar initial inoculums (0.1 OD at 670nm). Among all cultures treated with four different concentrations (0.5ppm, 2ppm, 4ppm, 6ppm) of $CoCl_2$, maximum growth was shown by cultures treated with 2ppm $CoCl_2$, among higher concentrations of $CoCl_2$ growth was reduced constantly. Optical density of treated cultures was gradually increased with increasing concentrations of $CoCl_2$, it reached to peak at 2ppm and among higher concentrations it reduced gradually (Figure - 1). Fresh weight and dry weight also show similar results(Figure 2-3). Growth rate calculated from dry weight also show similar results (Figure - 4), cultures treated with 2ppm $CoCl_2$ express highest growth rate, growth rate was maximum after 16 days of treatment in cultures treated with 2ppm $CoCl_2$, however among other cultures highest growth rate was found after three weeks of incubation. Growth rate was rose till third week of culturing after that it was reduced constantly.

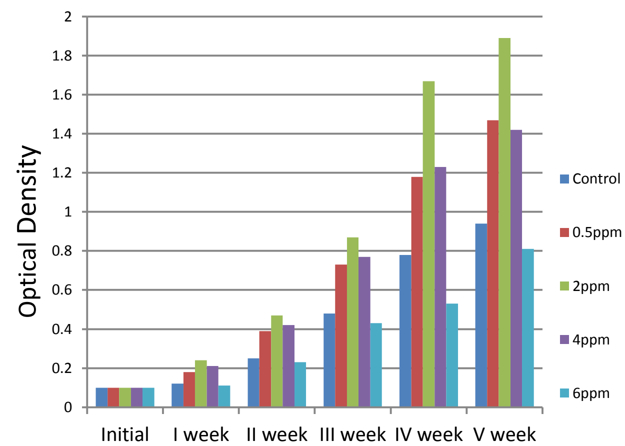

Figure 1 : Effect of different concentrations of cobalt chloride on growth (optical density) of *Chlorella vulgaris*.

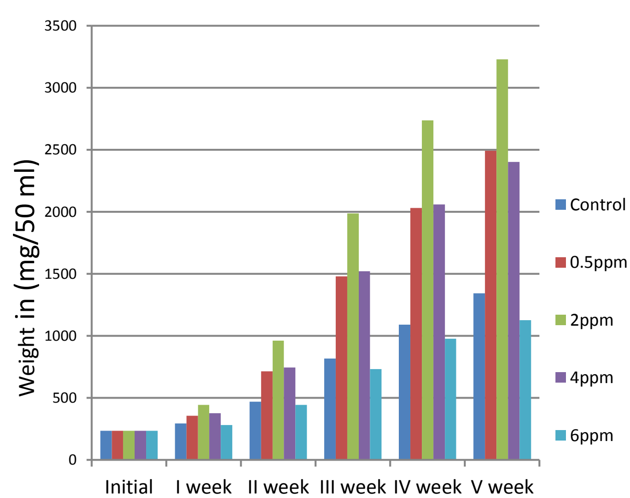

Figure 2 : Effect of different concentrations of cobalt chloride on growth (fresh weight in mg/50ml) of *Chlorella vulgaris.*

The pigment content of *Chlorella vulgaris* also correlates with growth of algae in treated cultures. Chl a and Chl b content was increased with increasing concentrations of $CoCl_2$ (Table 1-2). Highest amount of Chl a and Chl b was found in cultures treated with 2ppm $CoCl_2$, among higher concentrations pigment content was reduced gradually, but among highest concentration of heavy metal chlorophyll was inhibited by metal toxicity. Results of carotenoid estimation were also related to chlorophyll estimation (Table 3).

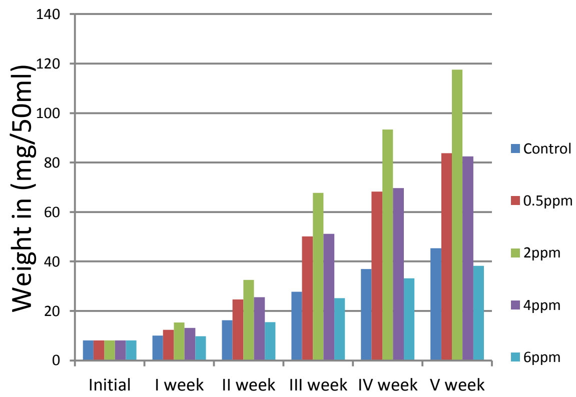

Figure 3 : Effect of different concentrations of cobalt chloride on growth (dry weight in mg/50ml) of *Chlorella vulgaris*

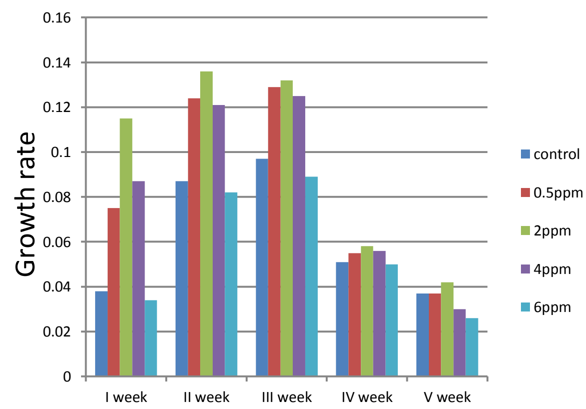

Figure 4 : Effect of different concentrations of cobalt chloride on g of *Chlorella vulgaris*

Table 1: Effect of different concentrations of cobalt chloride on chlorophyll – a (%) of *Chlorella vulgaris*, values are mean ±standard deviation (n=3).

Time	Control	0.5ppm	2ppm	4ppm	6ppm
Initial	.92±.016	.92±.029	.92±.008	.92±.014	.92±.024
I Week	1.01±.016	1.20±.017	1.42±.025	1.02±.043	.99±.029
II Week	1.17±.014	1.27±.051	1.49±.051	1.19±.014	1.13±.033
III Week	1.22±.049	1.31±.059	1.57±.029	1.29±.075	1.21±.021
IV Week	1.29±.045	1.38±.022	1.61±.037	1.39±.045	1.27±.022
V Week	1.33±.067	1.43±.037	1.69±.072	1.47±.059	1.24±.034

Table 2: Effect of different concentrations of cobalt chloride on chlorophyll – b (%) of *Chlorella vulgaris*, values are mean ±standard deviation (n=3).

Time	Control	0.5ppm	2ppm	4ppm	6ppm
Initial	.32±.016	.32±.024	.32±.016	.32±.012	.32±.016
I Week	.372±.053	.383±.051	.412±.006	.431±.007	.375±.075
II Week	.379±.097	.410±.047	.496±.004	.503±.006	.394±.070
III Week	.413±.042	.431±.054	.542±.006	.521±.005	.416±.080
IV Week	.427±.041	.453±.043	.567±.008	.516±.005	.421±.057
V Week	.436±.076	.462±.042	.576±.008	.523±.017	.434±.021

Table 3: Effect of different concentrations of cobalt chloride on carotenoids (%) of *Chlorella vulgaris*, values are mean ±standard deviation(n=3).

Time	control	0.5ppm	2ppm	4ppm	6ppm
Initial	.121±.014	.121±.012	.121±.020	.121±.008	.121±.023
I Week	.126±.016	.132±.020	.135±.035	.129±.037	.125±.016
II Week	.127±.026	.137±.022	.146±.031	.139±.030	.132±.030
III Week	.134±.034	.147±.032	.153±.040	.146±.018	.141±.023
IV Week	.139±.026	.151±.033	.163±.047	.156±.034	.141±.018
V Week	.143±.027	.158±.030	.167±.037	.162±.036	.145±.024

Highest carotenoids were found in cultures treated with 2ppm $CoCl_2$. Carotenoids have slightly greater tolerance than chlorophyll for heavy metals. The present results show resemblance with those obtained by M. M El-Sheekh et al (2003) who reported that 0.5ppm and 1.5ppm cobalt resulted in slight increase in growth and biopigments of *Nitzchia perminuta*, but among higher concentrations of heavy metal growth and pigment content reduced constantly. On the other hand growth promotion at low $CoCl_2$ concentrations may be due to Co^{2+} substitution for Zn^{2+} in some metalloenzymes as reported by Price and Morel (1990). These results show a significant increase in chlorophylls and carotenoids of *Chlorella vulgaris* at lower concentrations (0.5 and 2ppm) of $CoCl_2$. However higher concentrations (4 and 6ppm) of cobalt chloride were associated with progressive reduction in pigments. In this regard, De Filippis et al. (1981) reported that reduction of chlorophyll a content is a common symptom of heavy metals toxicity.Because *Chlorella vulgaris* have high nutritional and pharmaceutical values the objective of this research was to determine the effect of heavy metal pollutants on growth and biopigments of algae, \research data also helpful to determine the tolerance rang of *Chlorella* for $CoCl_2$. It was mentioned in results that moderate concentrations (2ppm) of heavy metals were best for growth and biopigments but high concentrations (6ppm) absolutely inhibit the growth and biopigment synthesis. So slightly increased concentration of cobalt is helpful for growth of algae in water bodies but high concentrations of that HM show negative influence on biomass and pigments of *Chlorella vulgaris*.

Acknowledgements

The authors wish to express their gratitude to Dr. Anirudh rishi and Akhilesh joshi for usefull discussions to improving our presentation.

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