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Toxicity of Cadmium (Cd) on microalgal growth, (IC50 value) and its exertions in biofuel production

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ABSTRACT

This study evaluated the IC_{50} value of cadmium against four different strains of microalgae. *Chlorella sorokiniana* was able to tolerate 300 mg/L of cadmium. The lipid productivity increased by 6% at 50 mg/l of Cd (II) stress. The decrease in biomass productivity was recorded with increasing concentration of Cd. The results showed that chlorophyll contents, chlorophyll a (Chl a) and chlorophyll b (Chl b) gradually decreased on increasing the concentration of Cd over 100 mg/l. The FAME composition of *C. sorokiniana* cultivated under Cd (II) stress and control medium were analyzed to determine the quality of the biodiesel produced. The major fatty acids present in the TAGs of the treated microalga were C10:0, C12:0, and C15:0.

Keywords:*Cadmium; Stress;Microalgae; Metabolites; Growth; Biofuel.*

1. INTRODUCTION

Cadmium (Cd) is the most widely known toxic and hazardous heavy metal environmental pollutant that leads to appalling illnesses in humans including cancer, kidney dysfunction, liver damage and bone diseases [1; 2; 3; 4; 5; 6]. The major causes pertaining to Cd toxicity due to its uncontrolled discharge in the environment by different industries [7].

Thus, Cd toxicity is one of the major environmental concerns globally that has attracted the attention of various researchers to tackle it by utilizing microbial flora such as fungi, bacteria, lignocellulosic materials and microalgae [8]. However, amongst all of the above microalgae have gained the most popular owing to their greater tolerance to Cd in comparison to other organisms.

Microalgae have evolved different intracellular and extracellular mechanisms to resist heavy metal toxicity and also discriminate between the essential and the non- essential heavy metals. This makes them a suitable practical option to tackle heavy metals in wastewaters [9; 10]. The use of both living and dead microalgae for removal of cadmium has been reported [11; 12; 13]. The efficient uptake of Cd has been reported in*Phormidium sp*. and *Spirullina sp.*[14].

Microalgae based biofuels have also been projected as world energy security against diminishing fossil fuels. This is because of their simple cultivation requirements and high lipid concentrations of many microalgal species in comparison to the traditional crops used for biofuel production [15;16; 17]. In-fact some researchers have reported Cd to increase lipid productivity [18; 19] in different microalgae which makes it a multipurpose biological agent to solve the issue of heavy metal pollution as well as the energy crisis. Metal induced strain leads to modifications in the lipid profile of the microalgae *viz*., composition, chain length, cetane number, viscosity, Nox emissions, etc.[20; 21; 22; 23]. Thus, metal-induced stress can be deliberately introduced in order to modify the fatty acid composition in microalgae and produce biodiesel of desirable quality and properties [24].

Keeping in view the identification of the above-mentioned fact of such microalgal species could open up new avenues towards integrating microalgal bioremediation and biofuel generation that too with enhanced lipid yields. Also, the selected microalgae can be further cultivated directly on cadmium polluted wastewaters which is further going to cut down the cost for cultivation media.

An effective hybrid approach has been presented here for cadmium removal complementing with enhanced lipid productivity in oleaginous microalgae *viz.Chorella singular* is, *Chorella sorokiniana*, *Chorella minutissima*, and *Scenedesmus abundans*) for biofuel generation. All the microalgae were first analyzed for their cadmium tolerance capabilities and were simultaneously assessed for the cadmium-induced stressed in lipid productivity. The findings of the study indicate this hybrid method as a cleanup approach pertaining to a sustainable environment.

2. MATERIALS AND METHODS

2.1. Materials. The four microalgal species *viz*., *Chorella singular Chorella sorokiniana*, *Chorella minutissima* and *Scenedesmus abundans* were available in Uttaranchal University, Dehradun, Uttarakhand, India.

2.2. Microalgae cultivation, Growth, Cd IC_{50} values determination.

The microalgal species were first cultivated in 500 ml flasks containing Bold's Basal Medium (BBM) [6] for 7 days at 24°C with cool white fluorescent light. Flasks were shaken manually after

regular intervals of time. The heavy metal tolerance of each of the four microalgae species was determined against $CdCl_2$. The stock solution of $CdCl_2$ (10mg/ml) was prepared and then diluted according to the requirement. After 96 h of microalgae cultivation growth of four microalgae was measured using a spectrophotometer the O.D was measured at 686nm. The IC_{50} value is that Cd concentration that reduces the microalgae cell viability by 50% as compared to microalgae cultivated in BBM. The maximum IC_{50} 0f Cd was recorded in *Chlorella sorokiniana* with an IC_{50} value of 300 µg/ml. The IC_{50} value is that Cd concentration which reduces the microalgae cell viability by 50% as compared to microalgae cultivated in BBM after 96h of cultivation [25; 26; 27].

$$\text{Percent inhibition} = \frac{\text{Microalgal cell in control medium} - \text{Microalgal cell in Treated medium}}{\text{Microalgal cell in control medium}} \times 100$$

The IC_{50} value was calculated using linear interpolation analysis and Microsoft Excel 2010.

The further study we have chosen the *Chlorella sorokiniana* to analyse the different parameters of microalgae.

2.3. Estimation of photosynthetic pigments.

For estimation of photosynthetic pigments 5ml of the microalgal culture was centrifuged at 5500 rpm for 5 min on 1oth day. For estimation of pigments 5ml of the microalgal culture was taken on the 10th day of cultivation and centrifuged at 5000 rpm for 5 min [6]. The obtained biomass was suspended in the 3 ml methanol and allow tostand at 45 °C for 30 min. Centrifuged, recorded the absorbances in supernant and evaluated the pigments according to Kumar et al., [6]. subtracting at 750 from other Absorbencies. Chlorophyll a (Chl a), chlorophyll b (Chl b) and Carotenoids (Car) were determined using the formulas given by Lichtenthaler, [28]

2.4. Estimation of Dry Cell Weight (DCW) and biomass productivity.

Microalgae growth was measured spectrophotometrically by taking the absorbance at 686 nm every second day for 14 days and biomass productivity was calculated as given by [29]. The microalgal cells were harvested by centrifugation at 8000 rpm for 10 min and the cells were dried overnight at room temperature. Further DCW was recorded using a digital weighing balance. Biomass productivity was determined using the following equation [30].

Biomass productivity (mg/L/D) = Change in DCW (g/L)/cultivation time (D)

2.5. Estimation of lipid yield and transesterification of fatty acid.

Lipid was extracted using the protocol described by Bligh and Dyer (1959) method [31]. Lipid yield (%) was calculated using the following equations:

Lipid yield = (Final lipid extracted- Initial lipid)/DCW.

Total lipid was transesterified into biodiesel using mechanic H_2SO_4[15]. Fatty acid composition in biodiesel was analyzed using GC-MS (GC-MS, Agilent) protocol described by Kumar et al., [15]. Biodiesel properties were calculated using BiodieselAnalyzer© Version 2.2.

2.6. FTIR analysis.

FTIR analysis microalgal cell cultivated in control and treated medium was done according to protocol provided by Arora et al., [32]. FTIR spectra were acquired using FTIR (FTIR 6700, NICOLET) in the range of 400–4000 cm^{-1}[33].

2.7. Statistical analysis.

All the microalgal cultures were grown in triplicates (n=3) ($p<0.05$). The results have been presented as mean (± S.D.).

3. RESULTS AND DISCUSSION

3.1. IC_{50} value and growth of microalgae.

All four microalgal species were found tolerant to $CdCl_2$ with different IC_{50} values (50-300 µg/ml) for the same.

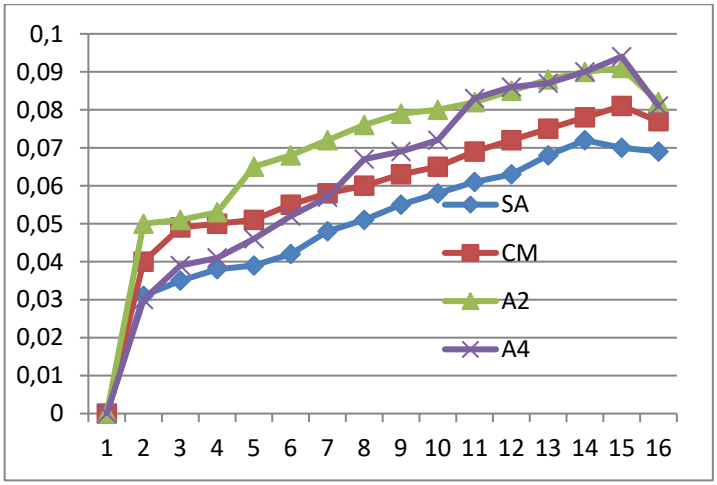

Figure 1. Growth of microalgal four species under Cd (II) Stress (100 mg/l). *A4-Chorella sorokiniana, Chorella minutissima* and SA-*Scenedesmus abundans, CM- Chorella minutissima, A2- Chorella singularis.*

The maximum IC_{50} value to Cd was recorded in *Chlorella sorokiniana* with an IC_{50} value of 300 mg/l. During the cultivation of microalgae, it also displayed the fastest adaption amongst the four microalgae with the shortest lag phase of 03 days amongst the four microalgal species (Fig 1). The growth was also uniform throughout the cultivation duration with a gradual increase in the number of cells.

3.2. Effect on photosynthetic pigments.

A decrease in all the photosynthetic pigments was recorded in *C. sorokiniana* cultivated in the presence of Cadmium (Cd) as compared to those cultivated in non-Cd containing medium (Table 1). The Chl a content decrease from 2.842 to 0.470 µg/mg while carotenoids concentration increase from 0.453 to 0.128 µg/mg at 50 mg/l of Cd. In past studies, researchers reported that Cd stress damage the biosynthesis of chlorophyll [34; 35; 36].

Table 1. Effect of Cd on pigments composition.

Photosynthetic Pigment (µg/ml)	Cd Conc. (µg/ml)			
	control	30	100	300
Chl a	2.842	0.470	1.383	0.923
Chl b	2.804	0.599	1.052	0.418
Car	0.453	0.128	0.286	0.222

3.3. Effect on biomass productivity and lipid yield.

Biomass productivity was decreased in Cd treated cell (300mg/l) (112 ± 0.01 g/L/d) on 5th day. 2 fold 2.6 fold decreases in *C. sorokiniana* biomass reported under Cd (300 mg/l) stress (Table 2). Increase in 6 % of lipid content under Cd treated algal cell at 50 mg/l as compared to the control. The increase in lipid content under metal stress conditions is common in algae cells [37; 38].

Under stress, the condition leads to less growth and algal cell metabolism shifts towards the synthesis of triacylglycerol [39; 40].

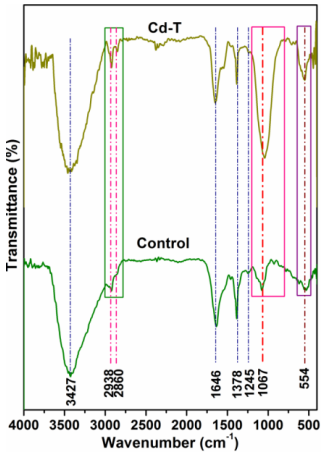

Figure 2. FTIR graph of A-Cd (II) treated (100 mg/l) and B-control *Chlorella sorokiniana*.

Table 2. Biomass productivity and Lipid yield under Cd stress.

Parameter	Cd concentration			
	Control	50	100	300
Biomass	800 mg/l	626 mg/l	539 mg/l	329 mg/l
Lipid yield	26.25 %	32.5%	24.44%	20.40%

3.4. FTIR analysis.

Normal algal cell has high carbohydrate, protein and photosynthetic pigments as compared to Cd treated cell (Fig 2). High peak was observed in 2938 cm^{-1} means Cd treated cell increase the lipid. 1067 cm^{-1} region showed high peak means Cd treated cell there is increase in carbohydrates content also.

3.5. FAME composition.

The feasibility of microalgal biomass cultivated for detoxification of Cd and its effect on biodiesel production was evaluated by analyzing the total lipid profile and fatty acid profile and compared to BBM (Fig. 3A and 3B). Microalgae lipids can be majorly categorized into structural/polar and storage/non-polar lipids [41].The FAME composition of *C. sorokiniana* cultivated in Cd stress cell and BBM medium was analyzed to determine the quality of the biodiesel produced. The major fatty acids present in the TAGs of the treated microalga were C10:0, C12:0, and C15:0 (Fig. 3B, Table 3 and 4).SFA (%) composition was good in both the biodiesel obtained from Cd treated and control microalgal biomass (table 5). PUFAwas not reported in Cd treated biomass biodiesel. The value of cetane number was good in both the diesel. High cetane number is good for complete combustion and smooth functioning of engine.

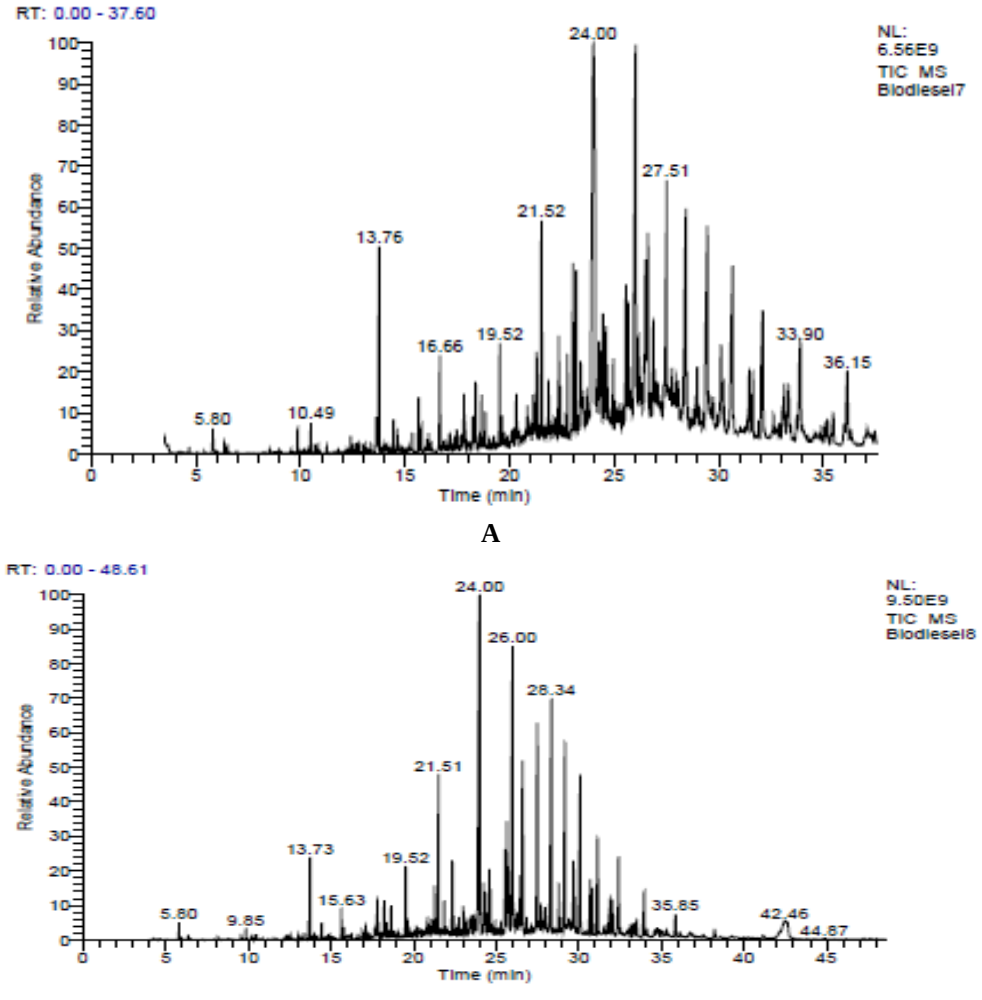

Figure 3. GC-MS graph of (A) Cd (II) treated (100 mg/l) and (B) control *Chlorella sorokiniana* biodiesel.

Table 3. GC-MS profile of biodiesel of microalgae cultivated in control medium**.**

Compound Name		Area %	RT
Nonanoic acid, methylester	C9	019	14.62
Decanoic acid, methylester	C10	0.56	18.37

Compound Name		Area %	RT
Dodecanoic acid, methylester	C12	0.22	20.98
Hexadecanoicacid,methyl ester	C18	12.15	24.01
Heptadecanoic acid,methyl ester	C17	0.77	24.95
Pentadecanoic acid,methyl ester	C15	7.38	26.02
9,12-Octadecadienoicacid (Z,Z)-, methyl ester	C18:2	0.97	26.54
Eicosanoic acid, methylester	C20	0.25	27.73
Docosanoic acid, methylester	C22	0.29	29.74

Table 4. GC-MS profile of biodiesel of microalgae cultivated under Cd stress.

Compound Name		Area %	RT
Decanoic acid, methylester	C10	2.65	21.50
Dodecanoic acid, methylester	C12	12.65	23.99
Heptadecanoic acid,methyl ester	C17	0.22	24.94
11-Octadecenoic acid,methyl ester	C18:1	1.33	25.71
Pentadecanoic acid,methyl ester	C15	8.49	25.99
Eicosanoic acid, methylester	C20	0.25	27.71

Table 5. Biodiesel properties of Cd treated andcontrol algal biomass.

Biodiesel Properties	Cd Treated	Control
SFA: Saturated Fatty Acid (%)	21.810	21.620
MUFA: Mono Unsaturated Fatty Acid (%)	1.330	0.000
PUFA: Poly Unsaturated Fatty Acid (%)	0.000	0.970
DU: Degree of Unsaturation	1.330	1.940
SV: Saponification Value (mg/g)	59.180	47.870
IV: Iodine Value	1.196	1.757
CN: Cetane number	138.258	159.921
LCSF: Long Chain Saturated Factor	0.250	6.760
CP: Cloud Point (°C)	-4.992	-4.992
PP: Pour Point (°C)	-12.240	-12.240

4. CONCLUSIONS

The IC_{50} values obtained was 300mg/l maximum for *C. sorokiniana*. An increase in lipid content by 6% was recorded in algal cells cultivated at 50mg/l concentration of Cd. Further increase in concentration decreased the lipid content. A decrease in photosynthetic pigments ovr 100 mg/l concentration of Cd was noted . The findigs of this study will help develop protocols to remediate Cd from contaminated water and the biomass can be used in biodiesel production.

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