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Research Article

ACUTE TOXICITY ON GROWTH AND CHLOROPHYLL *a'* CONTENT OF DIATOM *ODONTELLA AURITA*

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ABSTRACT

The effect of cadmium on the growth, chlorophyll *a* content and biomass of diatom, *Odontella aurita* was studied. The acute growth inhibition toxicity tests were conducted by exposing the subcultures on six different concentrations of $CdCl_2$ *viz.* 11, 20, 38, 72, 137, 261µg l^{-1} for 96 hrs. The cell density and chlorophyll *a* contents were decreased with increasing concentrations. Maximum cell density of 9.21 ± 0.01× 10^4 cells ml^{-1} at control and the minimum of 3.25 ± 0.14 × 10^4 cells ml^{-1} at the concentration of 261 ppb were found. The Chl *a* concentration of 0.11 ±0.01 pg. $cell^{-1}$ at concentration of 20 ppb were found and 0.02 ± 0.01 pg $cell^{-1}$ found in the highest concentration of cadmium. The 96 h median lethal concentration (LC_{50}) of cadmium 60.50 ± 0.26 µg l^{-1} and 10% of lethal concentration 21.305 ± 1.6 µg l^{-1} for *Odontella aurita* based on total recoverable cadmium concentration. In this 95% confidance limits of lower and higher value was 44.4 ± 0.61 and 83.1 ± 0.58 µg l^{-1}. The biomass was also decreased with increased cadmium concentration due to cadmium induced stress.

Keywords: Cadmium, *O. aurita* , Growth, Chlorophyll *a*, LC_{10}, LC_{50}

INTRODUCTION

The exposure of marine algae to metals, produces several different toxic effects, some elevated levels of heavy metals elicit deleterious impact on algae and cyanobacteria. Toxicity of heavy metals to algae primarily results from their binding to sulphydryl groups in proteins or disruption of protein structure or displacement of an essential element (De Filippis and Pallaghy, 1994). Many chemical contaminants, including organochlorine compounds, herbicides, domestic and municipal wastes, petroleum products and heavy metals are now recognized to have adverse affects on ocean environments, even when released at low levels (Haynes and Johnson, 2000); Pinho *et al.,* 2003). Metal contamination in aquatic environments is a widespread environmental problem although the impact of high concentrations of metals on the coastal phytoplankton community has been more difficult to detect. It is thus critical to understand the physical-chemical and biological behaviors of metals.

Cadmium (Cd) is a priority pollutant, and its toxicity is mainly related to binding the sulfhydryl groups of proteins or displacement of essential metals in metalloenzymes, It is generally thought that Cd is a toxic metal with no biological function, except in some marine diatoms (Lane and Morel, 2000; Lane et al., 2005) . Microalgae are primary producers at the base of the aquatic food chain and one of the first groups to be affected by metal pollution and the bio indicator of various pollutants (Sampathkumar and Kannan, 1998; Stauber and Davies, 2000; Ashok prabu *et al.*, 2008; Karthikeyan *et al.*, 2010). It is particularly difficult to accurate determination of cadmium content in phytoplankton which is the first stage in the marine food web. GESAMP (1985) reported that green and red macroalgae do not accumulate cadmium above about 2.1 g/g, while brown algae contained a wide range of cadmium (0.2 - 26 µg/g), which is believed to reflect ambient concentrations. Microalgae are the most important and basic component in marine and fresh water ecosystems (Li *et al.,* 2006).

In general the growth inhibition test with phytoplankton is not much simple because maintenance of culture and conduct of experiments are very critical. So, there has not much development in this field in worldwide besides OECD, USEPA and EPS have been developed a growth inhibition test procedures (OECD, 2002; OECD, 1984 and USEPA, 2002). The toxic level of heavy metal affects a variety of processes in plants (Maksymiec, 1997; Siedlecka *et al.,* 2001). One of the major consequences is the enhanced production of Reactive oxygen species (ROS), which damage cell membrane nucleic acids and chloroplast (Somashekaraiah, 1992; Tewari, 2002).

But their inhibitory effects on other parameters of marine algae particularly marine phytoplankton have not been thoroughly studied. Furthermore, information is very limited on the effects of cadmium on marine phytoplanktonic organisms from Indian waters. Hence, the effect of cadmium on the centric diatom *Odontella aurita* was studied in the present investigation. Thus the present study was carried out to find out the optimal nutrient concentrations of cadmium for its well growth, photosynthetic pigment and biomass production.

MATERIALS AND METHODS

Isolation, Identification and cultivation of diatoms:

The marine centric diatom, *Odontella aurita* was collected from Vellar estuary, Southeast coast of India (Latitude 11º29'N and Longitude 79º46'E). It was identified by manually, isolated and maintained at Algal Culture Laboratory, CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, India, Following the methods described by Anderson (2005). The strain was maintained with filtered natural seawater enriched with F/2 Guillard media recipe.

All the experiments were conducted in 5 litter conical flasks with 2500 ml of exponentially grown algal culture with the initial cell density of 3.21 ± 0.12 $\times 10^4$ cells mL^{-1}. The culture was exposed to different concentrations of cadmium *viz*. 11.0, 20.0, 38.0, 72.0, 137, 261.2µg $l^{-1}$for 96 hours and respectively for seven days. Each concentrations and experiments were replicated for three times.

Growth Inhibition Test:

Most algal bioassays are growth inhibition tests, which measure the decrease in cell division rate or final cell biomass after a few days in the presence of toxicant, compared to controls in dilution water. The range finding tests were conducted for 48 h before definitive test. The stock solution of cadmium was prepared in Milli-Q ultra pure water using its metallic salt of cadmium chloride dihydrate (Merck, India (Pvt.) Ltd.). The algal cell density (growth) was estimated at every 24 hours interval, the cell density was calculated by conventional method (100 µL) of *Odontella aurita* culture was made up to 1 ml with using lugol's iodine solution. 10µL of diluted sample was placed on a glass slide exactly at the meeting point of plus mark. The slide was mounted on the binocular microscope and the cells were counted. The results were expressed as cells mL^{-1} of culture.

During the experiment, the standard growth inhibition test procedures were followed by OECD 2002 and USEPA 2002. Other data process and graphs were plotted using SPSS 11.0 and anova and MS-Excel software. The dissolved cadmium concentrations of seawater and test solution were analyzed following the method of Grasshoff et al. (1999) in Atomic absorbtion of spectrophotometer (AAS), as such as through the APDC-MIBK extraction methods was used as 1000 ml of sample pour in a two literes of pre cleaned separating funnel and added 10ml of 2% of APDC solution and shake well for 5min. after 30 second, added 15 ml of MIBK solvent and shake well for again 20 minuts forther allowed the solution to stand for 15 minutes for separation, after separation transfer the aqueous layer (lower layer) into a pre-cleaned separating funnel and the MIBK extract (organic layer) into a pre-cleaned 100ml capacity separating funnel, and the collected aqueous solution is again extracted by adding 5ml of 2% APDC solution and 10ml MIBK solvent, following the steps mentioned in before, after again extracted the aqueous layer discarded and collect the MIBK extract into the separating funnel containing the first (MIBK) extract. The extract the metal now by adding 0.1ml of concentrated nitric acid into the combine MIBK extract , shake well and allow 10 minuts to separate the layers. Add 9.9 ml of Milli-Q water, shake vigorously for 5 minuts and allow the

phases to separate and then collected the metal containing acid layer (lower layer) into a 25ml volumetric flasc repeat the same procedure likewise as 0.1ml of concetrated nitric cid into the combine MIBK ectract shake well and allow 10 min to separate the layers and added 9.9 ml of Milli-Q water fartharly and make up to the mark with milli –Q water immediately stored in a pre-cleaned plastic bnottle and quantify in AAS. The abiotic parameters were maintained at 25 ± 1º C of temperature, 30‰ of salinity and 4500 ± 500 Lux of light intensity with 12:12 h light and dark condition during the experiment.

Chlorophyll 'a' estimation:

Chlorophyll was estimated by the modified method of Strickland and Parsons (1972). Five mL of acetone was added to 2 mL of algal culture and vortexed for 1 minute and kept in refrigerator at 4ºC for 24 hrs under dark condition. Then the samples were centrifuged at 5,000 rpm for 10 min and the supernatant was read at 630, 645 and 660 nm using UV–Vis Spectrophotometer (Perkin-Elmer Lamda 25). Raw acetone was used as blank, 10 mL aliquots of algal cultures were collected by centrifugation at 12,000 rpm for 10 min. Concentration of chlorophyll *a* was then calculated using the extinction values as described by Strickland, (1972).

Doubling time and growth inhibition was calculated by Growth rate:

The growth rate was calculated using the following formula (OECD, 2002),

$$\text{Growth rate } (\mu) = \frac{N_x - N_0}{t_x - t_0}$$

where,

N0 – Number of cells in time zero

Nx - Number of cells in time x

t0 – starting time (0)

tx – time X (in days)

Doubling time:

The doubling time was calculated by the following formula,

$$\text{Doubling time} = \frac{N_0 \times 2}{N_t} \times t$$

where,

N0 – Number of cells in time zero

Nt – Number of cell in time t

t – Time in hours

The results were presented in doubling time (DT) in hours.

Percentage of growth inhibition:

Percentage of growth inhibition was calculated by the formula,

$$\text{Percentage of Growth Inhibition} = \frac{\mu_{Control} - \mu_{Concentration}}{\mu_{Control}} \times 100$$

Median inhibitory concentration (IC_{50}) and 10 % Inhibition concentration (IC_{10}):

Growth rate and percentage of growth inhibition were calculated by mean cell density and time interval. The percentage of growth inhibition with respect to control in each concentration was used to calculate IC_{10} & IC_{50} with the use of Probit analysis software.

RESULTS

The *Odontella aurita* grew in all cadmium concentrations assay except 261µg l^{-1} concentration. The maximum cell density of 9.20 ± 0.01 and 8.88 ± 0.04 × 10^4 cells ml^{-1} were reached in control cultures and test cultures respectively with 11 µg l^{-1} of cadmium. The cell density was gradually decreased at end of the test period with increasing concentrations of cadmium at 96 hours (Table 1). The minimum cell density of 3.25 ± 0.14 × 10^4 was observed in 261 µg l^{-1} (Table 1) due to the reducing of growth rate while increasing growth inhibition (Fig. 1 & 2). The calculated 96-h IC_{10} and IC_{50} of value respectively 21.305 ± 1.6 and 60.50 ± 0.26 µg Cd L^{-1} were derived in acute tests based on measured cadmium concentrations (Table 2).

Cadmium (µg L^{-1})	Initial cell density (×10^5 cells mL^{-1})	Final cell density (× 10^5 cells mL^{-1})	Chlorophyll 'a' (pg. $cell^{-1}$)	Bio mass (mg Wet wt. /mL^{-1})
Control	3.20 ± 0.14	9.20 ± 0.01	0.10 ±0.01	1.189 ± 0.12
11		8.88 ± 0.04	0.11 ± 0.01	0.947 ± 0.03
20		8.39 ± 0.16	0.09 ±0.00	0.894 ± 0.01
38		7.60 ± 0.01	0.08 ± 0.00	0.670 ± 0.21
72		6.35 ± 0.21	0.06 ± 0.01	0.583 ± 0.10
137		4.08 ± 0.18	0.03 ±0.01	0.336 ± 0.10
261		3.25 ± 0.14	0.02 ±0.01	0.196 ± 0.04

Table 1: Cellular densities and cellular content of chlorophyll *a* obtained in cultures of ***O. aurita*** after 96 hrs exposure to cadmium at different concentrations.

Acute Test	
Nominal Concentrations (µg L^{-1})	**Measured Concentrations (µg L^{-1})**
11	06 ±1.3
20	10 ±1.0
38	22 ±1.2
72	46 ±1.5
137	107±1.2
261	221±2.0

Table 2: Total dissolved cadmium concentrations (µg L^{-1}) (Mean ± SD) of acute tests in test solutions after 96 hrs of exposures.

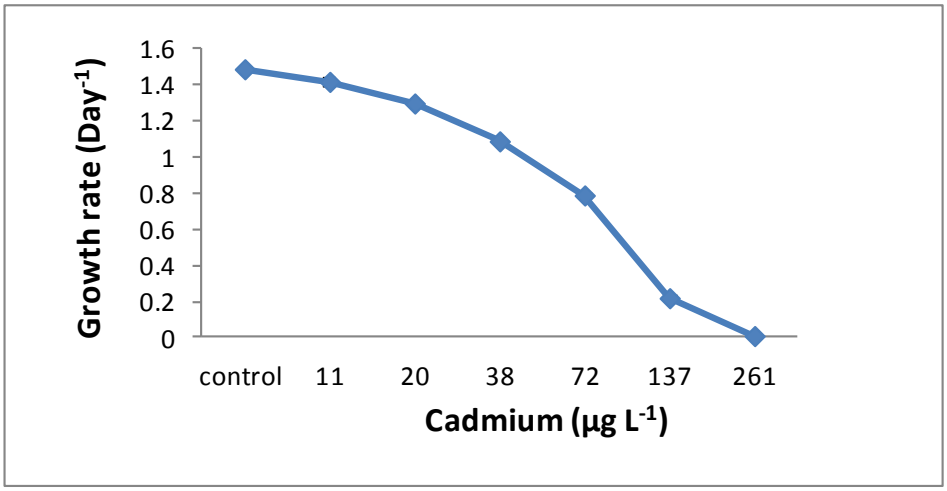

Figure 1: Dose-Response curve of acute growth rate toxicity test on O.aurita for 96 hours exposure to Cadmium

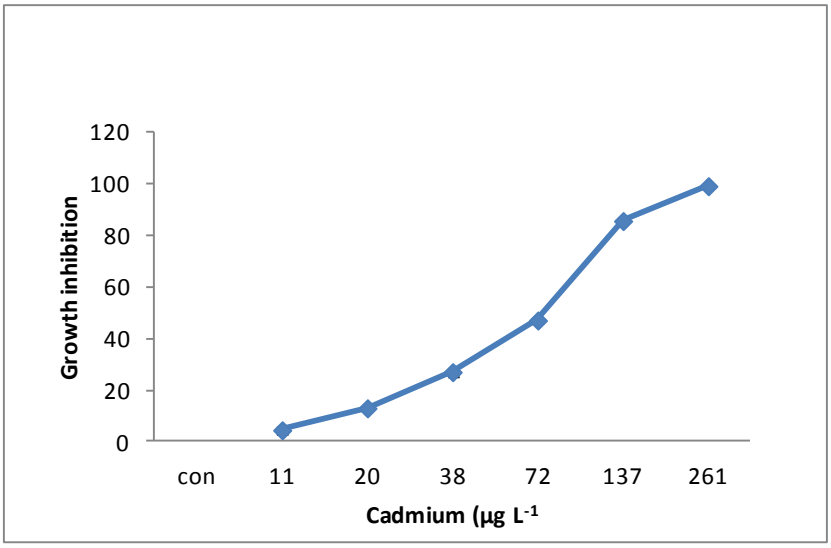

Figure 2: Dose- Response curve based on growth inhibition of cadmium on ***O.aurita*** in acute growth inhibition tests after 96 hrs exposure

The similar results were extended by Thongraar *et al.* (1995) who studied cadmium toxicity on Dunaliella tertiolecta and reported that the IC_{25} and IC_{50} values for reduced cell growth were 903 and 3,963 µg l^{-1} respectively. These results were lower than the values obtained for chlorophyll *a* content. The effect of harmful concentrations of cadmium on algae has already been determined by Gonzales (1995) and Rachlin *et al.* (1982).

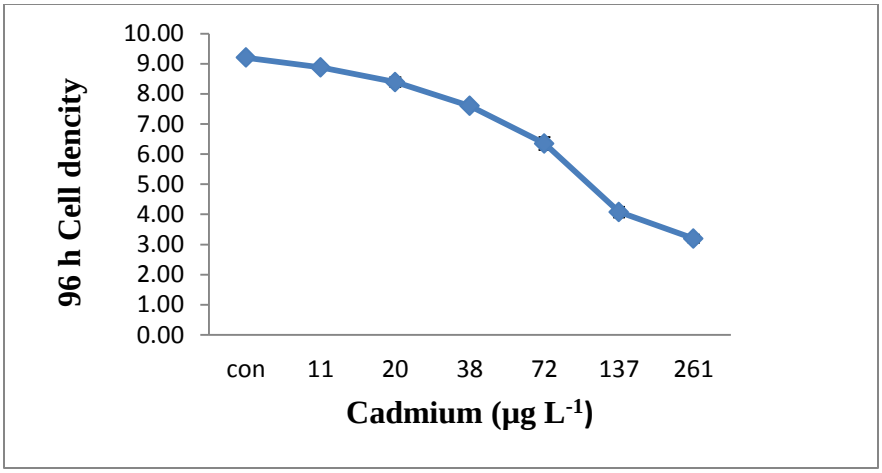

Figure 3: Dose- Response curve for cell density of *O. aurita* on cadmium exposure after 96 hrs exposure

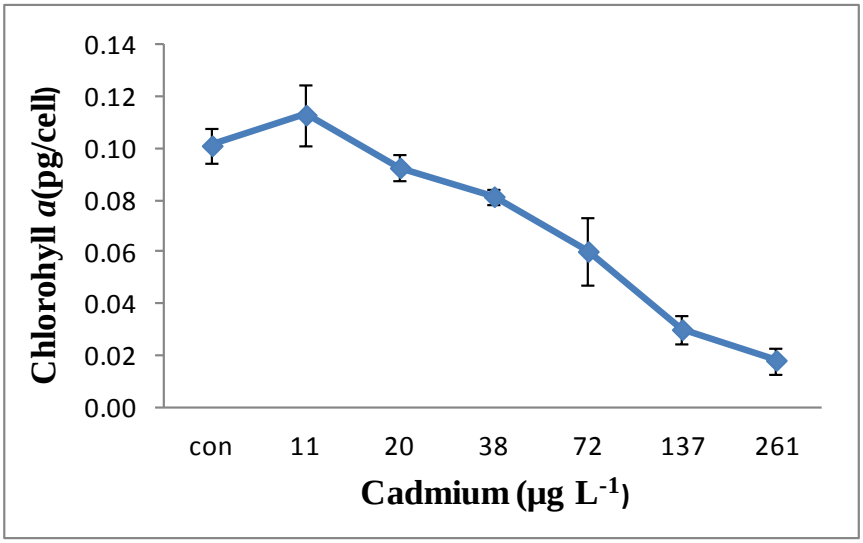

Figure 4: Dose- Response curve for chlorophyll *a* content of ***O. aurita*** on cadmium exposure after 96 hrs exposure

In the present study, the chlorophyll *a* content was decreased with increasing concentration of cadmium (Fig. 4). The low amount of chlorophyll *a* (0.018 pg.cell^{-1}) was observed in highest concentration of cadmium (261 µg l^{-1}) and higher amount of chlorophyll *a* (0.11 pg.cell^{-1}) was observed in first concentration(11 µg l^{-1}) and in control (10 pg.cell^{-1}), while compare the first concentration of Chl 'a' with control slightly increase of (Fig. 1). According to Kupper *et al.* (2002) at lower concentrations Cu^{2+} took over the functions of Mg^{2+} which showed elevated level of chlorophyll concentrations, at the higher concentrations the chlorophyll level was reduced because Cu^{2+} inhibits the synthesis of d-aminolevulinic acid and the protochlorophyllide reductase (Stiborova *et al.,* 1986), De Filippis *et al.* (1981) statement was proved with the reduction of chlorophyll *a* contents is a common symptom due to heavy metals toxicity. This may be attributed to the inhibition of reduction steps in the biosynthetic pathways of the pigment. Singh and Sing (1992) were also described the photoautotrophic growth of Nostoc calcicola was severely inhibited with

concurrent loss of photosynthetic pigments (phycocyanins > chlorophyll *a* > carotenoids).

The exposure to high metal concentrations may alter the plasma membrane permeability as a consequence of membrane functionality loss (Meharg, 1993; Payne and Price, 1999; Hernandez *et al.*, 1997. However, this was likely caused by the decrease in the specific cell growth rate and the increase in the cell size at a high-Cd level. Furnas (1978) has reported a significant decrease in growth rate in small cells of Chaetoceros curvisetum and others have observed more rapid division by larger cells recently derived from auxospores. The percentage of growth inhibition also showed (Fig. 2) in the present study was also confirmed that the cadmium concentration have influenced on the toxicity of *O. aurita*.

CONCLUSION

The present study is contributed to understand the effect of cadmium on the growth, chlorophyll *a* content and Biomass content of a diatom *Odondella aurita.* The cell density was decreased with increasing concentration of cadmium. (Fig.3). While the metal concentration was increased the biomass has been also reduced and the growth inhibition was increased with decreasing of average daily growth rate (Fig. 1&2). The chlorophyll a was significantly reduced with increasing concentrations of cadmium in this regard De Filippis et al. (1981) reported that reduction of chlorophyll a content is a common symptom of heavy metals toxicity. but the present study indicated that application of low Cd concentrations (11µg L^1) to *O. aurita* cultures led to significant increases in chlorophyll *'a'* although biomass reducing at the end of the test period as while increasing Chl '*a*', but biomass reducing, there is cell thickness only high with low cell division, the results agree with by Xuan Li et al., (2012). On the other hand, while increases in Cd concentration (20, 38, 72, 137 and 261) for *O. aurita* caused reduction in the Chlorophll "a"content (Fig. 4). According to the results, the cadmium was highly affected the growth rate and Chlorophyll '*a*' concentration of the marine diatom *O. aurita.* The results concerning the effect of different acute concentrations of Cd on the diatom studied here are in agreement with those of El-Sheekh et al., 2003 and Csatorday et al. (1984) who reported that the effect of Co2+, at low concentration need to growth of algae although at high concentration it will be inhibit to growth of algae. The present studies results was sported by P. Karthikeyan et al., (2011)

In contrast, reduction of photosynthetic pigments by the heavy metals also indirectly influences the photosynthesis. Photosynthetic pigments are easily measurable and frequently used to determine stress for regulatory purposes. Heavy metals decrease the chlorophyll content of algae, the chlorophyll a/b ratio, pheophytin levels but increase protochlorophyll levels and carotenoid/chlorophyll ratios in the majority of cases (De Filippis and Pallaghy, 1994). There are few reports that showed the enhancement of pigments after the exposure to metals (Deviprasad and Deviprasad, 1982), photosynthetic pigments were found to be reduced under the excessive concentrations of Hg (Rai *et al.*; 1981), Cu (Gross *et al.;* 1970), Cr (Corradi *et al.*; 1995), Ni (Xylander and Braune, 1994), Cd (Conway, 1978) and Zn (De Filippis and Pallaghy, 1976)

Based on the results, the marine centric diatom, *O. aurita* is more sensitive to cadmium. Chlorophyll 'a' and biomass were affected at higher concentration of cadmium. Further studies have to be carried out with

other trace elements and common pollutants for the environmental safety issues. The present investigation results can be useful for the derivation of species mean acute value, the development of seawater quality criteria.

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