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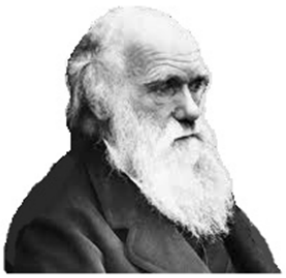

World News of Natural Sciences

An International Scientific Journal

WNOFNS 31 (2020) 48-57 EISSN 2543-5426

Cadmium Toxicity Towards Marine Diatom *Thalassiosira* sp. and its Alteration on Chlorophyll-a and Carotenoid Content

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ABSTRACT

Cadmium is one of the non-essential metals that have toxic effects on aquatic organisms, including diatoms. Diatoms play significant important roles in the aquatic environment, hence, the presence of cadmium will have a significance growth inhibition to its abundance. In this paper, we tried to clarify the effects of cadmium on growth, chlorophyll-a and carotenoid contents of the diatom *Thalassiosira* sp. Growth inhibition of the phytoplankton was determined following exposure for 96h to several different concentrations of cadmium solutions in an experiment adapted from ASEAN-Canada CPMS II. Our work shows that IC50 of growth and chlorophyll-a was around 0.32 mg/L and 0.914 mg/L, respectively. In addition, Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) were 0.18 mg/L and 0.1 mg/L, respectively. In conclusion, cadmium inhibits the growth, as well as the photosynthetic pigment contents of *Thalassiosira* sp.

Keywords: Cadmium, Carotenoid, Chlorophyll-a, *Thalassiosira* sp

1. INTRODUCTION

Human industrial activities such as manufacturing and the practice of agriculture have the tendency to discharge chemical waste that harm the surrounding environment [1]. Industrial

(Received 12 April 2020; Accepted 02 May 2020; Date of Publication 03 May 2020)

waste, notably heavy metals, are toxic to almost every acquatic life forms, leading to lethal effects even in very small concentrations [2]. According to the World Health Organization, there are 13 metals that have harmful effects to human and environment, one of these metals is cadmium [3].

Cadmium is commonly used in industry. For example, cadmium is employed in ceramic processing [4], as the Ni-Cd cathode in electric batteries [5] and in metallurgy [6]. This creates numerous amounts of cadmium-containing waste that is mostly discharged into the aquatic environment. Cadmium can inhibit the growth of certain organisms, particularly, microalgae by interrupting the photosynthetic process through bringing about the degradation of the chlorophyll bodies [7]. Moreover, it must be underlined that through bioacumulation, it can harm humans [8].

Thalassiosira sp. is one of the important and most abundant microalgae species that are collectively termed *Bacillariophyceae* or diatoms [9, 10]. *Thalassiosira* sp. is a primary producer in the aquatic environment, like other diatom species, and is known to be sensitive to heavy metal contamination and is capable of reducing its concentrations by producing metallothioneins and phytochelatins under heavy metal stress [11]. This makes them potentially ideal test organisms in toxicity assessment due to cost-effectiveness and abundance in the aquatic environment [12].

The growth of *Thalassiosira* sp. is an essential parameter in the aquatic environment and has significant correlation with their ability to perform photosynthesis. The photosynthetic process is helped by two pigments, Chlorophyll-a, which supports the photosynthetic process by transforming the light harvested by another important pigment, carotenoid, into photochemical energy [13, 14]. Carotenoid had an additional role as a photoprotecting agent, preventing excessive light from damaging the cell and Reactive Oxygen Species (ROS) from being created [15]. For some phytoplankton, carotenoid can also act as an antioxidant agent [16].

A clear profile of metals toxicity, in this case, cadmium, is needed to better understand the impact created by its discharge into the environment. Here, we experimentally measure the effects of cadmium by calculating the inhibition concentration or IC50, Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) to test and understand the effects of the toxicant on the diatom. Endpoints of this test, including cell density and content of chlorophyll-a and carotenoid of *Thalassiosira* sp., will be the basis data for the calculations.

2. MATERIALS AND METHODS

2. 1. Phytoplankton Cultivation

This research followed the protocol of American Standard Testing Material (ASTM) [17]. All of the glassware used were washed in 10% HNO_3 for 15 minutes and rinsed in distilled water and acetone in 3 repetition each to remove any heavy metal and organic material attached to the glassware. The washed glassware were then sterilized using an autoclave at 121 ºC and left to dry in the oven.

The culture of *Thalassiosira* sp. was obtained from the Laboratory of Mariculture, Research Center for Oceanography, Indonesian Institute of Science. The cultures of *Thalassiosira* sp. were grown in Erlenmeyer flasks containing seawater enriched with Walne

medium with EDTA (Ethylene Diamine Tetraacetic Acid-chelating agents). Cultivation temperature was maintained at 27 ºC. The cultures were given oxygen aeration, were covered in aluminum foil and were illuminated in continuous light. The components of Walne medium are listed in Table 1.

Table 1. Composition of Walne medium according to ASEAN Canada CPMSII [18].

Components	**Compositition**	**Amount in 100 mL distilled water**
Vitamin solution Materials	Vitamin B1	100 mg
	Vitamin B2	5 mg
Trace elements solution Materials	$ZnCl_2$	2.1 gr
	$CoCl_2$	2 gr
	$(NH_4)_6Mo_7O_2 \cdot 4H_2O$	0.9 gr
	$CuSO_4 \cdot 5H_2O$	2 gr
Nutrient solution Materials	$NaNO_3$	10.0 gr
	Na_2EDTA	4.5 gr
	H_3BO_3	3.36 gr
	$NaH_2PO_4 \cdot H_2O$	2.0 gr
	$FeCl_3 \cdot 6H_2O$	0.13 gr
	$MnCl_2 \cdot 4H_2O$	0.036 gr
	Vitamin solution Materials	10 mL
	Trace elements solution Materials	0.1 mL

The *Thalassiosira* sp. density was counted daily using a haemocytometer under microscope viewing towards the death phase, where the decline in cell growth is apparent. The exponential phase of phytoplankton was used for the growth inhibition tests of *Thalassiosira* sp. in the presence cadmium. Cells in the exponential phase were examined because the phytoplankton grows optimally in this phase.

2. 2. Growth Inhibition Test

The 4 days old exponential phrase phytoplankton were inoculated to perform the growth inhibition test in Walne medium with no presence of EDTA, and with initial density of 1×10^4

cell/ml. The stock solution of cadmium were prepared using $CdCl_2 \cdot H_2O$, Merck in deionized water. Water Quality Parameters were recorded (temperature 28.31 – 28.66 ºC, pH 7.15 – 7.31, salinity 33.0 – 33.2 ppt, dissolved oxygen 3.47 – 5.99 mg/L) to ensure only that the toxicant affects were responsible for any variance in the growth of the phytoplankton.

The toxicity tests were carried out for 96 hours using triplicate experimental procedures with different concentrations (0.1, 0.18, 0.32, 0.56, 1.0, and 1.8 mg/L) obtained from prior range finding testing. To prevent any contamination, all flasks were wrapped with aluminum foil and placed randomly in the incubation chamber with continuous illumination. Each day, all solution in flasks were shaken twice to prevent precipitation [19].

The result of this test are cell density measurements after the exposure of cadmium, and test were terminated each day by adding 0.9 mL of sample to a sterile vial, followed by the addition of 0.1 mL Lugol's solution to preserve it. Cell density were then estimated using a haemocytometer with the help of a microscope.

2. 3. Chlorophyll-A and Carotenoid Measurement of *Thalassiosira* sp.

After 96 hours, the phytoplankton cultures from each flask were filtered through a type of sartorius filter paper with pore size 0.45 µm to obtain the chlorophyll-a and carotenoid extract for analysis. This was then folded and wrapped up carefully in aluminum foil to prevent penetration of light. The filter paper was subsequently preserved under refrigeration after being labeled until further analysis was performed.

Intracellular analysis of pigment was performed by treating the filter paper/ *Thalassiosira* sp. extract with 7 mL acetone. The samples were then centrifuged at 3000 rpm for 30 minutes to separate the filtrate from pigments. The results of centrifugation were then placed inside a cuvette for absorbance testing.

The test were carried out using a UV-Vis spectrophotometer at the wavelengths of 664, 647, 630, 510 and 480 nm to determine the chlorophyll-a and carotenoid concentration. The concentration of total chlorophyll-a and carotenoid was calculated by applying the following calculation [20]:

Ca (µg/mL) : 11.85 E664 – 1.54 E647 – 0.08 E630
C (p) (µg/mL) : 7.6 (E480 – 1.49 E510)

2. 4. Data Analysis

Algal cell densities in each flasks were calculated at the end of the test for the growth inhibition by means of the calculation below [21]:

$$I = \frac{C - T}{C} \times 100\%$$

where: C was the average cell densities in the control and T was the average cell densities in the treatments.

Further analysis was carried out using ICPIN software to estimate the values of 96-h toxicity test IC50. One-way ANOVA (TOXSTAT software) was then applied to determine the significant effects of Cd and control of cell densities and chlorophyll-a and carotenoid content of *Thalassiosira* sp.

3. RESULT AND DISCUSSIONS

3. 1. Toxicity of Cadmium (Cd) to Growth of *Thalassiosira* sp.

After 96 hours exposure to the heavy metal cadmium, a sharp decrease in the cell density of *Thalassiosira* sp. became apparent. This was in alignment with the increase of inhibition percentage in each treatment as compared to control. Figure 1 shows that the density of *Thalassiosira* sp. decreased consistently as the amount of cadmium concentration increased.

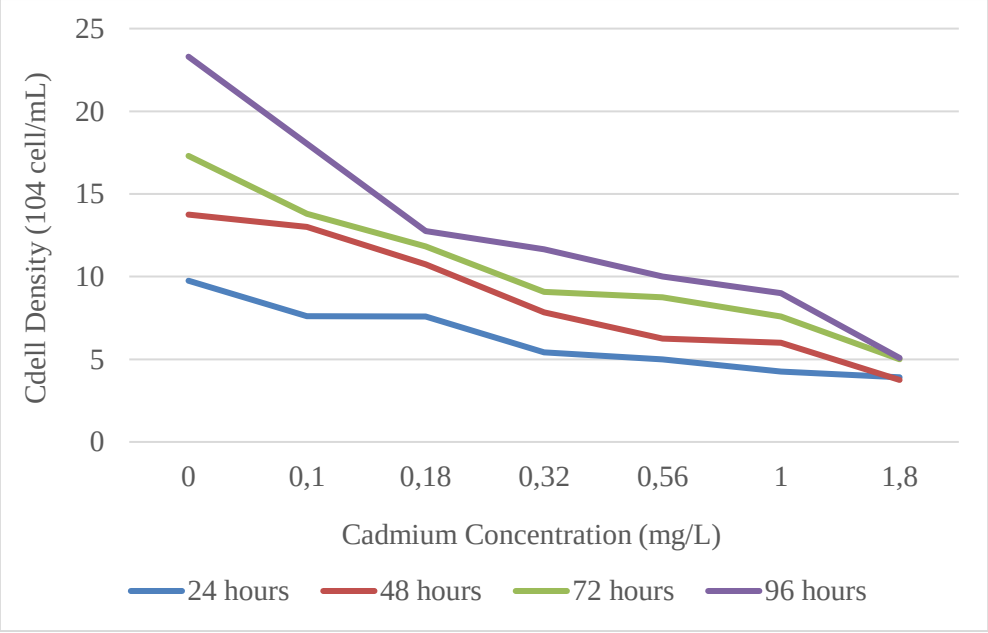

Figure 1. Growth Curve of *Thalassiosira* sp. after Cadmium Exposure

The density of the control treatment after 96 hours is 23.3×10^4 cells/mL. The decreases of concentration were significant when compared with the starting concentration of 0.18 mg/L, according to the NOEC and LOEC results. The NOEC value for growth inhibition of cadmium was 0.1 mg/L, whereas the LOEC value was 0.18 mg/L. Meanwhile, the IC_{50} value based on ICPIN data analysis was 0.32 mg/L. The percentage of growth inhibition in this concentration was 49.95%.

In general, heavy metal disturbance brings about damage to the cell membrane [22-25]. Heavy metals are able to hinder cell growth through two mechanisms, passive and active absorption. Passive absorption happens when interaction occurs between the metals and the cell membrane, while active absorption arises by heavy metal contamination being transported through the cell membrane into the cytoplasm. Passive absorption might come about if there is an excessive amount of metal concentration outside the cell than inside. In active absorption, outside energy is needed to perform the transportation of the metals [26].

With regard to heavy metal contamination, the growth inhibition of phytoplankton is dependent on the amount of heavy metal ions bound to the cell's surface, as well as the chemical characteristics of the heavy metal ion [26, 27]. Cadmium is a lipophilic heavy metal. This means that the metal is soluble in lipids. This characteristic helps cadmium to bind with protein in the

cell's membrane, hence cadmium can be absorbed into the diatom's cell [28, 29]. Moreover, cadmium can bind with the cell's DNA and nucleic protein, thus interrupting DNA replication by inducing apoptosis within cell cycle. At lower concentrations, cadmium will bind with cell protein to decrease DNA repair ability and will activate protein degradation processes [26].

3. 2. Chlorophyll-*a* and carotenoid content of *Thalassiosira* sp.

The analysis of chlorophyll-*a* and carotenoid content were carried out after 96 hours of toxicity testing. Here we found that cadmium negatively affected the content of *Thalassiosira* sp. pigments according to quantification results using an UV-Vis Spectrophotometer. Figure 2 shows the result of chlorophyll-*a* and carotenoid content investigation of *Thalassiosira* sp. after the absorbance test and related calculations.

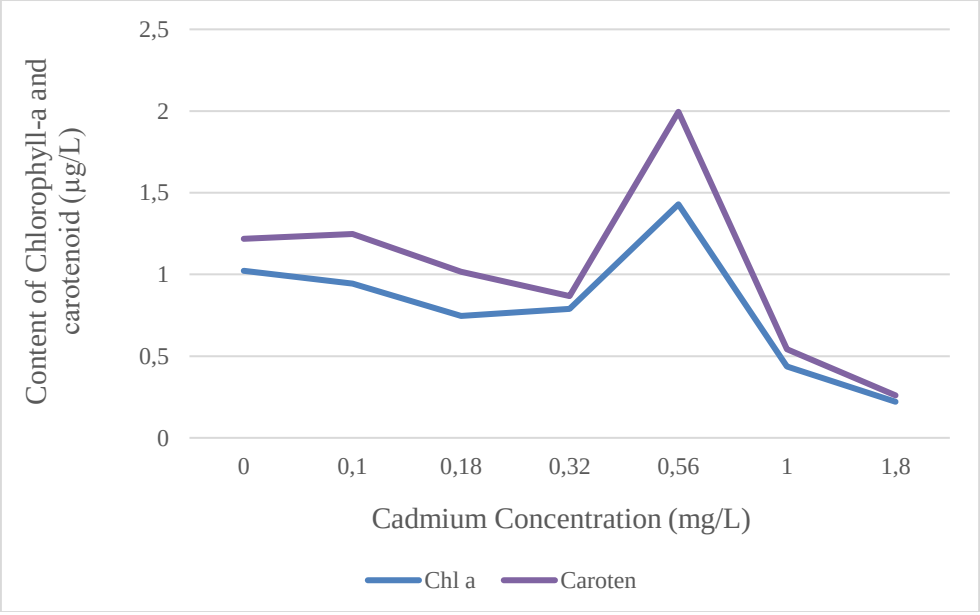

Figure 2. Chlorophyll-*a* and Carotenoid Content of *Thalassiosira* sp. after 96 Hours of Cadmium Exposure

It can be seen in the graph above, that the content of chlorophyll-*a* and carotenoid is almost correlated to the cell densities, with the exception of concentration at 0.56 mg/L. Although there were a sudden rise of pigment content in the 1 mg/L concentration, from the pattern we can see that the more that cadmium is added into the solutions, the less content of chlorophyll-*a* and carotenoid is recorded. The IC_{50} value of chlorophyll-*a* was calculated to be approximately 0.914 mg/L. Thus, it can be concluded that cell density served as a better parameter of toxicity since it was more sensitive compared to chlorophyll-*a* content. This is in agreement with previously published research [30-32].

Moreover, published research has shown that cadmium will inhibit the biosynthesis of chlorophyll-*a* and carotenoid [33]. Herein, cadmium will enter the cell through the help of the cell metal transporter (Ca and Fe transporter) and inactivate the reaction center of Photosystem II [34]. In addition, the content of chlorophyll-*a* can be reduced due to chlorosis which is a

chlorophyll degradation process [35-38]. Chlorosis caused by cadmium can occur in two ways: First, through the inhibition of synthesis of the 5-amino-luvelinicacid enzyme which has an important role in chlorophyll biosynthesis [28]; Secondly, by changing the magnesium content in the middle of the cyclic ring of chlorophyll, thus interrupting carbon fixation in the Calvin cycle [39]. Furthermore, ionic forms of cadmium are also known to inhibit the photosynthetic process by degrading the thylakoid membrane. This degradation causes the inhibition of the chemical reaction within the photosynthetic process and decreases the amount of chlorophyll within the cell, which can lower the production of ATP and NADPH in photosynthesis. This last results in interruption of cell metabolic activities, thus inhibiting the growth of phytoplankton [40].

3. CONCLUSIONS

In summary, growth inhibiton of *Thalassiosira* sp. and cadmium concentration are correlated; the greater the concentration of cadmium, the less cell densities of *Thalassiosira* sp. obtained. In our research, the IC_{50} of growth inhibition after 96 hours of toxicity testing were 0.32 mg/L, while, LOEC and NOEC for cadmium is 0.18 mg/L and 0.1 mg/L, respectively. IC_{50} of chlorophyll-*a* content was also calculated at 0.914 mg/L. This number shows that IC_{50} of growth inhibition is more sensitive to cadmium exposure than pigment content.

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