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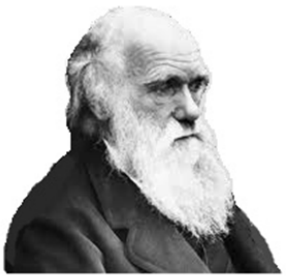

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Cadmium Toxicity Towards Marine Diatom *Thalassiosira* sp. and its Alteration on Chlorophyll-a and Carotenoid Content

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ABSTRACT

Cadmium is one of the non-essential metals which have toxic effects on aquatic organism, including diatom. Diatom has significant important roles on aquatic environment, subsequently the presence of cadmium will have a significance growth inhibition to its abundance. Here we tried to understand 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 concentration of cadmium solutions adapted from ASEAN-Canada CPMS II. Growth IC50 of growth and chlorophyll-a was calculated to be 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 also calculated to be 0.18 mg/L and 0.1 mg/L, respectively. In conclusion, cadmium inhibiting the growth, as well as the photosynthetic pigment contents of *Thalassiosira* sp.

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

1. INTRODUCTION

Human activities such as manufacture industries and agriculture have the tendency to discharge their chemical waste which will harm the surrounding environment [1]. Industrial

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waste, notably heavy metals are toxic to almost every life forms of aquatic organism, leading to lethal effect in very small concentration [2]. According to the World Health Organization, there are 13 metals which have harmful effects to human and environment, one of those metal are cadmium [3].

The usage of cadmium is well known among the industrial manufactures. For example, cadmium is being used in ceramic processing [4], Ni-Cd cathode in electric battery [5] and metallurgy [6]. This created numerous amount of cadmium-containing waste that mostly discharged into the aquatic environment. Cadmium can inhibit the growth of certain organisms particularly microalgae by interrupting the photosynthetic process by degrading the chlorophyll of microalgae [7]. Even more, through bioacumulation it can harm human creating several acute problems [8].

Thalassiosira sp. is one of important microalgae species which included in *Bacillariophyceae* or known as diatom. The clade is known as one ofthe most abundant type of phytoplankton in aquatic environment [9, 10]. *Thalassiosira* sp. is a primary producer in aquatic environment, like other diatom species. Furthermore, phytoplankton is known to be sensitive to heavy metal and capable of reducing its concentrations by producing metallothioneins and phytochelatins under heavy metal stress [11]. This makes them as a potential as test organism in toxicity assesment due to its cost-effectiveness and abundance in aquatic environment [12].

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

A clear profile of metals toxicity, in this case cadmium, is needed to better understand the impact created to 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 any effect of the toxicant to 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 autoclave in 121 ºC and dried in the oven.

The culture of *Thalassiosira* sp, obtained from Laboratory of Mariculture, Research Center for Oceanography, Indonesian Institute of Science and Walne medium were used to sustain its growth. The culture of *Thalassiosira* sp. were grown in Erlenmeyer flask containing seawater enriched with Walne medium with EDTA (Ethylene Diamine Tetraacetic Acid-

chelating agents) with cultivation temperature maintained at 27 ºC. The culture were given an oxygen aeration, covered in aluminum foil and were illuminated in continuous light. The components of Walne medium are listed in Table 1.

Table 1. Composisiton 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 haemocytometer under microscope towards the death phase, where the decline in cell's growth are apparent. The exponential phase of phytoplankton were used for the growth inhibition tests of *Thalassiosira* sp. in the presence cadmium. Cell in exponential phase were used due to the phytoplankton can grow optimally in this phase

2. 2. Growth Inhibition Test

The 4 days old phytoplankton, which were in the exponential phase, was inoculated to perform the growth inhibition test in Walne medium with no presence of EDTA 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. Condition of test water Water quality parameter 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 make sure only toxicant affects that responsible to the growth of phytoplankton.

Toxicity test carried out for 96 hours using triplicate experiment with different concentration (0.1, 0.18, 0.32, 0.56, 1.0, and 1.8 mg/L) obtained from prior range finding test. 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 shook twice to prevent precipitation [19].

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

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

The phytoplankton culture which have been kept for 96 hours from each flasks were filtered through a type of sartorius filter paper with pore size 0.45 µm to get the chlorophyll-a and carotenoid extract for analysis. It was then folded and wrapped up carefully in aluminum foil to prevent penetration of light. The filter paper was then preserved in refrigerator after being labeled until further analysis performed to prevent the solution from being damaged.

Intracellular analysis of pigment was performed by extracting the filter paper which containing *Thalassiosira* sp. with 7 mL acetone. The samples were then centrifuged in 3000 rpm for 30 minutes to separate the filtrate from pigments. The result of centrifugation then placed inside of a cuvette for analysis of absorbance test.

The test are carried out measure by UV-Vis spectrophotometer at the wavelength of 664, 647, 630, 510 and 480 nm to determine the chlorophyll-a and carotenoid concentration. of *Thalassiosira* sp. The concentration of total chlorophyll-a and carotenoid was calculated using 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 using 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 was used to estimate the values of 96-h toxicity test IC50. One-way ANOVA was then used to determine the significant effects of Cd and control of cell densities and chlorophyll-a and carotenoid content of Thalassiosira sp. The calculation of ANOVA was using the TOXSTAT software.

3. RESULT AND DISCUSSIONS

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

After 96 hour exposure to the heavy metal cadmium, a sharp decrease in the cell density of *Thalassiosira* sp. became apparent which aligned with the increase of inhibition percentage in each treatments compared to controls. Figure 1 shows that the density of *Thalassiosira* sp. was decreasing consistently as the amount of cadmium concentration increase in the test.

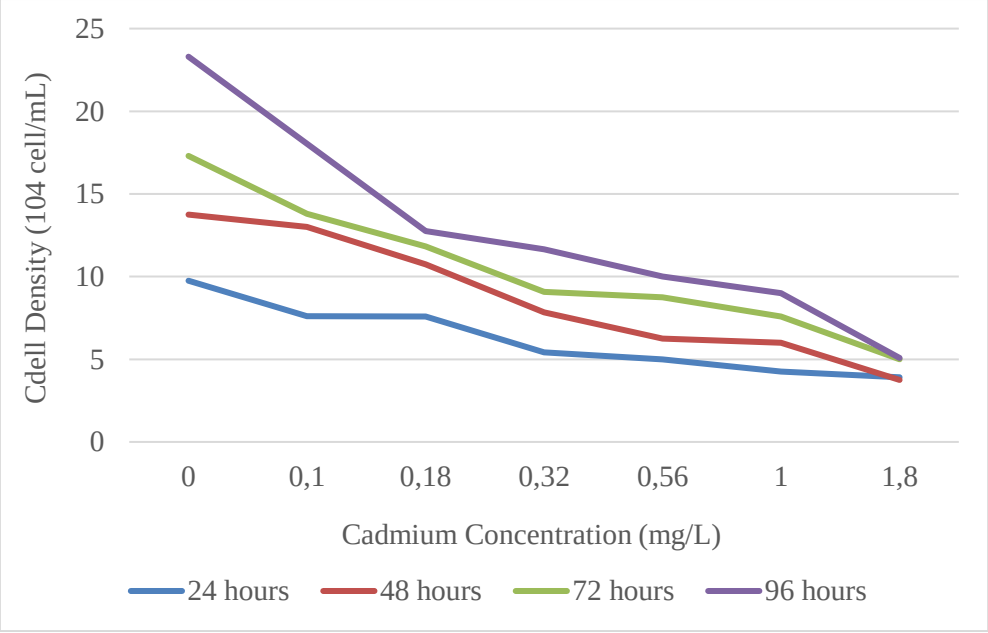

Figure 1. Growth Curve of *Thalassiosira* sp. after Cadmium Exposure

The graph above shows that as more cadmium concentration being used, the cell densities of *Thalassiosira* sp. decrease. This result occurs since the first time cell densities were measured. The densities of control treatment after 96 hours are 23.3×10^4 cells/mL. The decrease of concentration were significant starting concentration 0.18 mg/L according to the NOEC and LOEC result. 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 exactly at 0.32 mg/L. The percentage of growth inhibition in that concentration was 49.95%.

In general, damaged cell membrane was the result of heavy metal disturbance [22-25]. Also, heavy metals are able to hinder cell growth through two mechanism, passive and active absorption. Passive absorption happens when there are an interaction happens between the metals and the cell membrane while the active absorption arises by transporting it through cell membrane into the cytoplasm. Passive absorption might happen if there is an excessive amount of metal concentration outside the cell than inside. As for active absorption, an energy is needed to perform the transportation of the metals [26].

The growth inhibition of phytoplankton were dependent on the amount of heavy metal ion bounded to the cell's surface and chemical characteristic of the heavy metal ion [26, 27].

Cadmium is a lipophilic heavy metal which mean the metal is soluble in lipid. This characteristic help cadmium to bound protein in the membrane cells which makes cadmium absorbed into the diatom's cell [28, 29]. Also, cadmium can bind itself with DNA and nucleic protein which will interrupt DNA with inducing apoptosis within cell cycle meanwhile at lower concentration, cadmium will bind protein to decrease DNA repair ability and activating protein degradation [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 test. Here we found that cadmium affecting the content of *Thalassiosira* sp. pigments negatively according to quantification results using UV-Vis Spectrophotometer. Figure 2 shows the result of chlorophyll-*a* and carotenoid content of *Thalassiosira* sp. after the absorbance test and calculation.

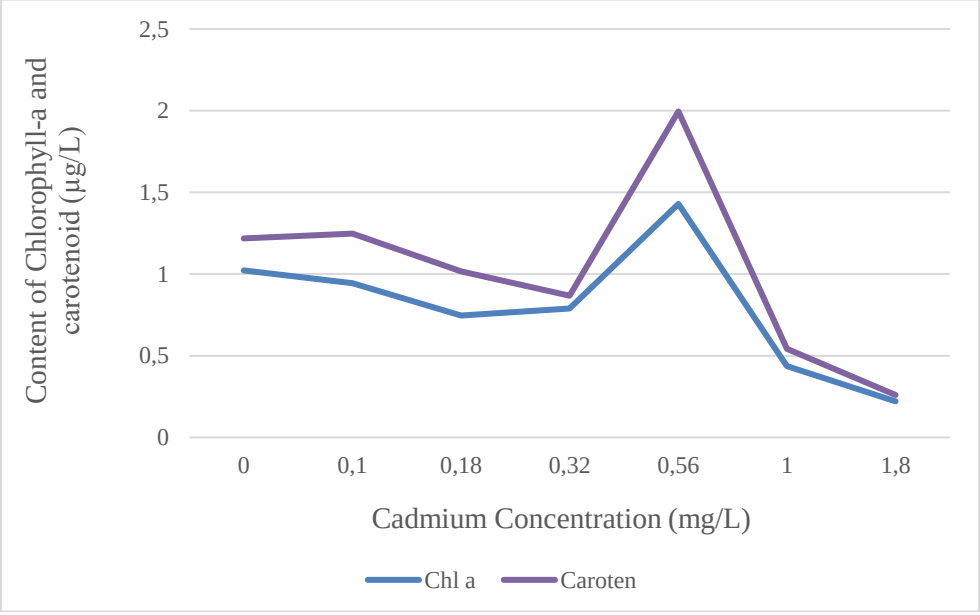

Figure 2. Chlorophyll-*a* and Carotenoid Contents of *Thalassiosira* sp. after 96 Hours of Cadmium Exsposure

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

Cadmium can affect the chlorophyll-*a* and carotenoid content negatively in *Thalassiosira* sp. According to published research, cadmium will inhibit the biosynthesis of chlorophyll-*a* and

carotenoid [33]. Cadmium will enter the cell through the help of metal transporter (Ca and Fe transporter) and inactivated reaction center of Photosystem II, which contain internal antenna domain bearing the chlorophyll, carotenoid and xanthophyll [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 5-amino-luvelinicacid enzyme which has an important role in chlorophyll biosynthesis [28]. Secondly, by changing the magnesium content in the middle of cyclic ring of chlorophyll thus interrupting carbon fixation in calvin cycle [39].

Ionic form of cadmium also known to inhibit the photosynthetic process by degrading thylakoid membrane. This degradation causing the inhibition of chemical reaction within photosynthetic process and decreasing the amount of chlorophyll, which can lower the production of ATP and NADPH in photosynthesis also resulting in interrupting with metabolic activities thus inhibiting the growth of phytoplankton [40].

3. CONCLUSIONS

In summary, growth inhibiton of *Thalassiosira* sp. and cadmium concentration are correlated as in, the more concentration of cadmium being used, the less cell densities of *Thalassiosira* sp. obtained. In addition, IC_{50} of growth inhibition in 96 hours toxicity test were valued at 0.32 mg/L. Meanwhile, LOEC and NOEC for cadmium is 0.18 mg/L and 0.1 mg/L respectively. IC_{50} of chlorophyll-*a* content also being calculated at 0.914 mg/L. This number shows that IC_{50} of growth inhibition is more sensitive to cadmium exposure than pigments content.

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