The Effects of Copper (Cu) and Cadmium (Cd) in 
*Chlamydomonas* sp. Growth

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Abstract

Heavy metal pollutant such as Cu and Cd may affect the life of aquatic organisms such as *Chlamydomonas* sp. A study aims to understand the effects of Cu and Cd present in the water toward the growth of *Chlamydomonas* sp. The toxicity of the heavy metals toward the growth of *Chlamydomonas* sp was tested following the standard methods of Asean-Canada Cooperative Program on Marine Science Phase-II (ACCPMS-II). Results shown that IC50-96 hours of Cu and Cd on the growth of Chlamydomonas sp. was 0.6 mgL-1, and 24.92 mgL-1 respectively. Data obtained proved that the presence of Cu and Cd in the water inhibit the growth of *Chlamydomonas* sp, and Cu is more toxic than Cd. The Cu toxicity was 42 times greater than that of Cd. The NOEC values for Cu was < 1 mgL-1, while that of Cd was 18 mgL-1. LOEC value was 1 mgL-1 for Cu, and 32 mgL-1 for Cd.

Keywords: Cu, Cd, Chlamydomonas sp., IC50-96 hour, NOEC, LOEC

1. Introduction

As a primary producer, phytoplankton plays a vital role and affect both the nutrient cycles of marine ecosystems, freshwater and other aquatic ecosystems [1]. This organism also responds to pollutants and control the concentration of pollutants in aquatic ecosystems, as well as removing the pollutant from the water. *Chlamydomonas* sp. is a species of phytoplankton that affected by the presence of pollutant in the water, including heavy metal pollutant. Heavy metals accumulate in the sediment and it may distribute to the water body due to environmental factors, such as wind, waves, currents and weather [2]. Heavy metals that enter the aquatic environment originated from various sources, namely natural source such as anthropogenic activities and industrial waste [3] and it is toxic for algae.

Copper (Cu) in the water commonly originated from industries, pesticides, antifouling paint, fungicides, and mining waste dumps [4]. While Copper (Cu) actually is an essential component of enzyme condensation such as oxidase enzymes and electron transport chains (such as plastocyanin) in plant [5], however at high concentrations or long exposure may inhibit the photosynthesis and negatively affect the metabolism [6]. Cd is non-essential, toxic in certain concentration and it may harm the organism [7]. As a major producer of the trophic environment on the organism, phytoplankton that accumulate heavy metals from the...
surrounding water may is transfer that metal to the higher trophic level organisms. So far, there are several experiments on the effects of Cu and Cd toward the growth of phytoplankton such as *Porphyridium* sp.[8], *Nannochloris* sp.[9]. In this study, *Chlamydomonas* sp. was used to test the toxicity of Cu and Cd in order to determine the pollutant concentration that negatively affects the growth and survival of the phytoplankton and compare which heavy metals are more toxic [10].

### 2. Method

The research was done in Marine Chemistry Laboratories And Ecotoxicology, Research Center for Oceanography, Indonesian Institute of Science, Jakarta Utara. Research on heavy metal toxicity test toward *Chlamydomonas* sp. based on the logarithmic/exponential phase. In this study growth curve and Definitive Test were observed. The curve was used to identify the exponential phases of *Chlamydomonas* sp. The IC50 value was obtained as a basis for determining the heavy metal concentration use in definitive test. The definitive test was done to understand the value of IC50, and obtaining the NOEC and LOEC values of Cu and Cd for *Chlamydomonas* sp [11].

#### Sterilization and Preparation

All equipment used in this studies was rinsed using non-phosphate detergent and soaked 15 minutes in a 10% HNO3 solution and rinsed with distilled water. Then the equipment were rinsed using acetone and distilled water again. Seawater used in this research has previously been filtered with 0.45 µm filter paper and sterilized with autoclave for ± 20 minutes, 1.5 Pa pressure at the temperature of 121°C [12].

#### Procedure

The CuCl2 and CdCl2 solution used in definitive test were 1000 ppm for stock solution. The solution was then diluted into 1000 mL with 1; 1.8; 3.2; 5.6; 10 mgL-1 Cu concentration and 18; 32; 56; 100; 180 mgL-1 Cd concentration, 3 replication. Water quality parameters measured were pH, DO, salinity and temperature [11].

#### Data Analysis

The percentage of inhibition or stimulation of phytoplankton growth were measured based on the number of cells in each treatment (T) and it is compared to the average of cells number in control (C), based on this equation:

\[
I\% = \frac{C - T}{C} \times 100\% \quad S\% = \frac{T - C}{C} \times 100\%
\]

The result of definitive test was calculated using ICPIN 2.0 program for IC50 [13] and analyzed with ANOVA and Dunnet Test that has already installed in TOXSTAT 3.2 program for NOEC and LOEC value [14].

### 3. Result And Discussion

#### *Chlamydomonas* sp. Growth Curve

The growth curve of *Chlamydomonas* sp. in the 17th day is presented in Fig. 1. During the day 0 to day 3, the *Chlamydomonas* sp. was in lag phase. In the day 4 to day 8, was in logarithmic phase/exponential. Day 9 to day 13 was on stationary phase. Day 15 to day 17 was on leading to death phase. The exponential phase is the stage which phytoplankton is
actively engaged cell division, it means that the number of phytoplankton increase [15]. The exponential phase is marked by rapid growth, constant metabolic activity and constant velocity. The growth of microalgae is depend on the food supply available [16].

In normal condition, the microalgae cultured should reach at least 1x106 cells/mL within 4-7 days after the inoculation [11]. If the microalgae species that do not achieve that density, that type of microalgae could not be used for toxicity test (96-h growth test). In this research, 4-7 days after the inoculation Chlamydomonas sp. reached 367.95 x 104 cells/mL or 3.68 x 106 cells/mL (Fig.1) and it means that this species can be used for toxicity test. In this study Chlamydomonas sp. can be used for testing Cu and Cd toxicity test within 4 days after inoculation.

![Graph showing growth curve of Chlamydomonas sp.](image)

**Fig 1. Growth Curve of Chlamydomonas sp.**

**Definitive Test**

The Definitive Test was assumed to be valid if the number of cells in control achieve ≥ 2 x 105 cells/mL within 96 h [11]. In this study Chlamydomonas sp. can be used for definitive test as it reached 2.49 x 105 cell/mL within 96-h in the control of Cu and Cd solution. The results of definitive test are presented in Fig 2.

![Graphs showing density and concentration vs. % inhibition](image)

**Fig 2. Result of Definitive Test**
After being exposed in Cu for 96 hours, there was decrease in the density of *Chlamydomonas* sp. However, there was increase in inhibition percentage in each treatment and these values were higher than that of the control. In the 3.2 mgL-1 Cu, phytoplankton cell increase, indicating that copper might be served as essential component for growth. In low concentration, copper is not toxic but will actually hamper the cell growth by becoming a cofactor for various growth enzymes. Copper is a component of plastocyanin in the electron transport chain in photosynthetic reactions [17]. At concentration 5.6 mgL-1 to 10 mgL-1, there was a constant decrease in density, it means that the toxic effects of copper affects the *Chlamydomonas* sp growth. The IC50 of Cu was 0.6 mgL-1 after 96 h of exposure in control. Copper (Cu) causes disruption in phytoplankton cell wall, by reducing the K+ ions concentration in the cell and disrupt electron transport[18]. The excess of Cu2+ will accumulate on the cell wall, absorbed into the cell and affect the enzyme by binding to the sulfhydryl group (-SH). The enzyme was disrupted and affects the reproductive ability.

In Cd control treatment, the cell density was 24.92 x 104 cell/mL. Inhibition percentage rise as Cd concentration increase, 36.12% at 18 mgL-1 to 95.42% at 180 mgL-1. The IC50 of Cd was 24.92 mgL-1 after 96 h of exposure in control. Exposure of cadmium to phytoplankton at high concentration will reduce metabolism and reduce thiol side of protein [19]. Result of previous study on 18 µgL-1 at *Chaetoceros gracilis*[18]; 0.18 mgL-1 at *Nitzschia* sp. [24] and 0.032 mgL-1 at *Porphyridium* sp. [8] shown that cadmium toxicity affects phytoplankton growth.

Based on the IC50 value, Cu toxicity on the growth of *Chlamydomonas* sp. was higher than that of the Cd, it was 0.6 mgL-1 and 24.92 mgL-1 respectively. The NOEC of Cu to the growth of *Chlamydomonas* sp. in this study was < 1 mgL-1, and LOEC was 1 mgL-1. While for Cd, the value of NOEC was 18 mgL-1 and the LOEC was 32 mgL-1. This value indicate that the maximum concentration of Cu and Cd present in the waters which have no negative effect on growth of *Chlamydomonas* sp. was < 0.1 mgL-1 for Cu and 18 mgL-1 for Cd. While the minimum concentration of Cu that hamper the growth of *Chlamydomonas* sp. was 1 mgL-1, and that of the Cd was 32 mgL-1. The IC50 value presented in Table 1 indicates that the toxicity of Cu is 42 times higher than Cd toward *Chlamydomonas* sp. Table 4 and 5 shown the comparison of NOEC and LOEC values of *Chlamydomonas* sp. and the NOEC and LOEC values of other microalgae.

**Table 1.** IC50-96 hours, LOEC 96 hours and NOEC 96 hours heavy metals Cu and Cd on the growth of Chlamydomonas sp.

| Heavy Metal | Standard | Value in ppm (mgL-1) | Value in ppb (µgL-1) |
|-------------|----------|----------------------|----------------------|
| Cu          | IC50     | 0.6004               | 600.4                |
|             | NOEC     | < 1                  | < 1000               |
|             | LOEC     | 1                    | 1000                 |
| Cd          | IC50     | 24.9167              | 24916.7              |
|             | NOEC     | 18                   | 18000                |
|             | LOEC     | 32                   | 32000                |

**Water Quality Parameters**

The standard [11] of water quality parameter values for conducting the toxicity test are presented in Table 2. While water quality parameter values obtained in this study are
presented in Table 3. Data on water quality parameters indicate that the water in *Chlamydomonas* sp. culture is fulfill the minimum requirement for phytoplankton test.

### Table 2. Recommended Conditions for Phytoplankton Growth Toxicity Test

| No. | Parameter                  | Test Condition                        |
|-----|----------------------------|---------------------------------------|
| 1.  | Test Type                  | Static                                |
| 2.  | Temperature                | 27 ± 1°C                              |
| 3.  | Photoperiod                | Continuous                            |
| 4.  | Light Quality              | Laboratorium Light Condition           |
| 5.  | Light Intensity            | 400 ± 40-foot candle                   |
| 6.  | Test flask size            | Erlenmeyer 250 mL                     |
| 7.  | Test solution volume       | 100 mL                                |
| 8.  | Age of stock phytoplankton culture | 4-7 hari                          |
| 9.  | Initial cell density       | 104 sel/mL                            |
| 10. | Replicate                  | 3                                     |
| 11. | Shaking Period             | Twice daily by hand                   |
| 12. | Nutrient                   | Walne’s non EDTA media                |
| 13. | Dissolution Factor         | 0.5                                   |
| 14. | Test Duration              | 96 h                                  |
| 15. | Effect measured            | Growth (cell counts)                  |
| 16. | End Point                  | IC50, NOEC and LOEC                   |
| 17. | Validity of Test           | Mean control density of 2 x 105 cells/mL |

### Table 3. Water Quality in the Definitive Test of Heavy Metal Cu and Cd

| Heavy Metal | Conc. mgL⁻¹ | Temp (0°C) | pH | Salinity (ppt) | DO (mg/L) |
|-------------|-------------|------------|----|----------------|-----------|
| Control     | 28.56       | 7.97       | 27.4 | 5.61           |
| 1           | 28.70       | 8.08       | 30.8 | 5.42           |
| 1.8         | 28.60       | 8.27       | 31.2 | 5.41           |
| Cu          | 3.2         | 28.66      | 7.30 | 26.2 | 5.64 |
| 5.6         | 28.66       | 7.35       | 28.8 | 5.55           |
| 10          | 28.67       | 7.45       | 28.7 | 5.40           |
| Control     | 28.30       | 7.72       | 30.2 | 5.80           |
| 18          | 28.25       | 8.27       | 30.2 | 5.48           |
| 32          | 28.37       | 7.22       | 27.1 | 5.41           |
| Cd          | 56          | 28.34      | 7.34 | 31.6 | 5.12 |
| 100         | 28.39       | 7.55       | 31.5 | 5.17           |
| 180         | 28.47       | 7.36       | 31.3 | 5.19           |

### Table 4. IC50, NOEC and LOEC Metal Cu Some Phytoplankton

| Species                  | IC50 (µg/l) | NOEC (µg/l) | LOEC (µg/l) | Duration of Test | References           |
|--------------------------|-------------|-------------|-------------|------------------|----------------------|
| *Tetraselmis* sp.        | 47          | 7           | 22          | 72 h             | Levy et al., 2007    |
| *Dunaliea tertiolecta*   | 530         | 42          | 8           | 72 h             | Levy et al., 2007    |
| *Nitzia closterium*      | 18          | 5.8         | 4.4         | 72 h             | Levy et al., 2007    |
| *Phaeodactylum tricornutum* | 8         | 1.5         | <1.5        | 72 h             | Levy et al., 2007    |
| *Micromonas pusilla*     | 1.2         | 0.6         | 0.3         | 72 h             | Levy et al., 2007    |
| *Minutocellus polymorpha* | 0.6       | 0.2         | <0.2        | 72 h             | Levy et al., 2007    |
| *Isochrysis galbana*     | 910         | -           | -           | 5 d              | Yap et al., 2004     |
| *Tetraselmis tetathele*  | 7400        | -           | -           | 72 h             | Satoh et al., 2004   |
| *Chaetoceros* sp.        | 88          | -           | -           | 72 h             | Debelius et al., 2005|
| *Chaetoceros gracilis*   | 63,75       | 10          | 17          | 96 h             | Suratno et al., 2015 |
Table 5. IC50, NOEC and LOEC Metal Cd Some Phytoplankton

| Species               | IC50 (µg/l) | NOEC (µg/l) | LOEC (µg/l) | Duration of Test | References                  |
|-----------------------|-------------|-------------|-------------|-----------------|-----------------------------|
| Chaetoceros gracilis | 2370        | 1000        | 1800        | 96 h            | Suratno et al., 2015 [18]  |
| Isochrysis sp.        | 490         | < 560       | 560         | 96 h            | Suratno et al., 2015 [18]  |
| Nitzschia sp.         | 159         | < 180       | 180         | 96 h            | Larasati, 2017 [24]        |
| Porphyridium sp.      | 93.9        | 32          | 56          | 96 h            | Margareta, 2018 [8]        |
| Nannochloris sp.      | 590         | < 100       | 100         | 96 h            | Wardani, 2018 [9]          |
| Chlamydomonas sp.     | 24912       | 18000       | 32000       | 96 h            | *In This Study              |

4. Conclusion
The growth of Chlamydomonas sp. was hampered by the presence of Cu and Cd. The IC50-96 hours of Cu is 0.6004 mgL-1, NOEC of < 1 mgL-1 and LOEC 1 mgL-1. While the IC50-96 hours of Cd was 24.92 mgL-1, NOEC of 18 mgL-1 and LOEC of 32 mgL-1. The toxicity of Cu is 42 times higher than that of Cd in hampering Chlamydomonas sp. growth.

5. Acknowledgments
The author would like to thank Dr. Dwi Hindarti, M.Sc as the supervisor during the author's research and Mr. Hardi as a technician of the Marine Chemistry and Ecotoxicology Laboratory, Research Center for Oceanography Research at LIPI, who has helped the writer complete this research.

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