

## Cu toxicity on growth and chlorophyll-a of *Chaetoceros* sp.

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**Abstract.** Phytoplankton is a primary producer in marine aquatic ecosystem. Their sensitivity to metal makes them important to study to predict the environmental impact of pollution. Copper is an essential nutrient for aquatic life as micronutrients on an organism but toxic at high levels. The focus of this study was to assess the toxicity of copper to *Chaetoceros* sp. on growth and chlorophyll-a content. The result shows that inhibition concentration (IC<sub>50</sub>) of copper on the microalgae, *Chaetoceros* sp. was 30.25 µg L<sup>-1</sup>. Growth of *Chaetoceros* sp. decreased 16.84% in 16 µg L<sup>-1</sup> and 81.97% in 44 µg L<sup>-1</sup>. Chlorophyll-a content decreased dramatically at 44 µg L<sup>-1</sup> compared to control. Increase of the cell size, deformation of cell wall and loss of setae were observed at higher concentration of copper.

### 1. Introduction

Phytoplankton is a primary component of the estuarine environment. They are at the basal level of food chain and a starting point of trophic transfer. In the open ocean, phytoplankton is the major source of primary production. By contrast, estuaries have a number of additional primary production sources, including detritus from salt marsh and terrestrial plants, benthic microalgae, macroalgae, and sea grasses [1]. Because of their key position as primary producers in aquatic ecological systems, microalgae are sensitive indicators of environmental change and are therefore important test species for the regulatory assessment of metals. However, the sensitivities of microalgae to metals such as copper vary over several orders of magnitude [2]. *Chaetoceros* sp. is suitable as test organism because they fulfill the requirement i.e growing fast, high sensitivity and easy to handle in the laboratory [3,4]. *Chaetoceros* sp. (Bacillariophyceae) is the largest genus and acts as a primary producer and is an important food for another biota, especially shrimp [5].

Copper is an essential nutrient for aquatic life. Essential metals such as Fe, Mn, Cu, Mo, Zn and Mg are important as micronutrients on an organism but toxic at high levels [6]. Copper in the marine environment may be derived from natural or anthropogenic source e. g. mining activities, industrial discharge, fertilizers, pesticides, algicide and antifouling paints [2]. The objective of this study is to determine the toxicity of copper to a single species of phytoplankton, *Chaetoceros* sp. and to study the Cu effect on morphology and chlorophyll-a.

### 2. Material and Method

#### 2.1. Experimental organism

The test organism, *Chaetoceros* sp., used for this study was easy to isolate from natural waters and to culture in laboratory. *Chaetoceros* sp. are fast growing species, high sensitivity and high abundance in the tropical ocean [3]. A culture was obtained from the Mariculture Laboratory, Research Center for Oceanography, Indonesian Institute of Sciences.



## 2.2. Culture methods

Walne culture medium was used to culture *Chaetoceros* sp. in the laboratory. The natural seawater was filtered using 0.45 $\mu$ m cellulose nitrate filter and then autoclaved for 20 min at 121°C. Walne medium was added to the sterilized seawater aseptically. The cultures were maintained in a 12:12 h light:dark cycle at 24  $\square$  C. All cultures were made aseptically under a laminar flow hood. All cultures were shaken twice each day by hand. Stock cultures were maintained in 250-ml Erlenmeyer flasks. All flasks were washed with nitric acid 10%, acetone and also rinsed de-ionized water [7].

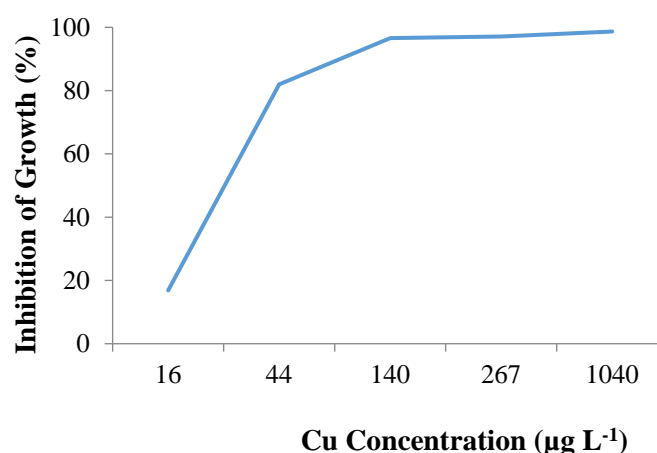
## 2.3. Phytoplankton Growth Toxicity Test

A 96-h growth rate IC<sub>50</sub> experiment exposing *Chaetoceros* sp. to copper was conducted using the following concentrations: 0; 100; 180; 320; 560; 1000  $\mu$ g L<sup>-1</sup>. Copper stock solutions 1000 mg l<sup>-1</sup> were prepared from copper chloride, CuCl<sub>2</sub>.2H<sub>2</sub>O. Five different copper treatments and controlled (filtered) natural seawater were prepared from the stock solutions in triplicate and distributed in 100mL quantity into 250-mL Erlenmeyer flasks. The actual concentration of copper was measured using Spectrophotometer HACH DR 2800 based on porphyrin method. Actual concentration tests were 0; 16; 44; 140; 267 and 1040  $\mu$ g L<sup>-1</sup>, respectively. The alga culture was then inoculated with 1 $\times$ 10<sup>4</sup> cells/mL to each test solution. 0.1 ml Walne medium non EDTA was added into each flask as a source nutrient for phytoplankton growth. All Erlenmeyer tubes were shaken twice a day by hand. After 96 h exposure, 0.9 ml of culture were sampled and mixed by 0.1 ml Lugol as a preservative compound. Test was valid if cell count in control after 96 h reached  $\geq$  2 $\times$ 10<sup>5</sup> cells. mL<sup>-1</sup>. This procedure followed the standard method of American Standard Testing Material with salinity and temperature modified according to tropical condition [7].

Cell counts were made with an improved Neubauer hemacytometer under standard bright-field microscopy. The 96 h IC<sub>50</sub>, i.e. the inhibitory concentration to reduce the growth rate by 50%, was calculated using linear interpolation (ICPIN version 2.0) [8]. Two ml of *Chaetoceros* sp. were subsampled at 96 h to analyze the morphology using Nikon DIAPHOT Microscope with DSLR Canon EOS 10D camera. Chlorophyll-a content was also analyzed after 96 h using Fluorometer Turner Trilogy type AU-10.

## 3. Result and Discussion

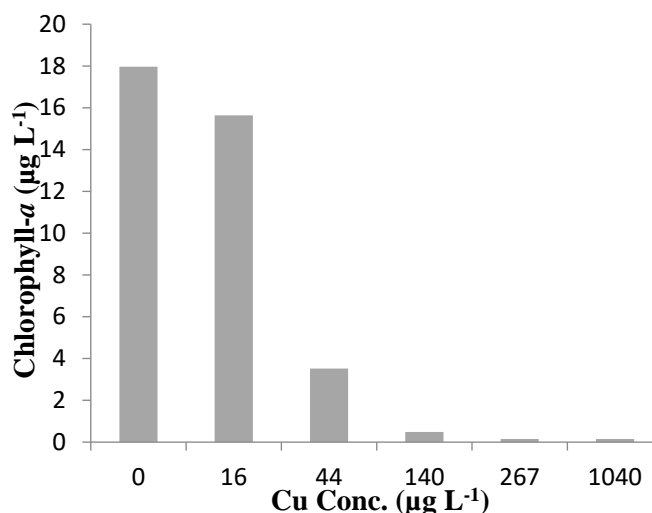
This phytoplankton toxicity test was valid because control in this study reached 12.7  $\times$  10<sup>5</sup> cell/ml after 96 hours exposure [7]. It fulfilled the criteria of validity test from ASTM. Growth inhibition of *Chaetoceros* sp. after 96 h exposure of copper is described in Figure 1.



**Figure 1.** Growth inhibition of *Chaetoceros* sp. after 96 hours exposed.

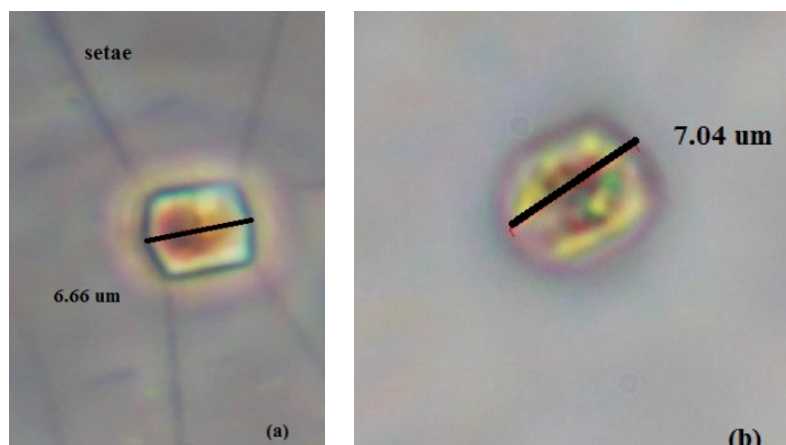
The growth of *Chaetoceros* sp. significantly decreased 16.84% in  $16 \mu\text{g L}^{-1}$  and 81.97 % in  $44 \mu\text{g L}^{-1}$ . From ICPIN analysis,  $\text{IC}_{50}$  96 h of copper to *Chaetoceros* sp. was  $30.25 \mu\text{g L}^{-1}$ . Previous study [9] reported that  $\text{IC}_{50}$  value of copper was  $31.80 \mu\text{g/L}$  for *Isochrysis* sp. and  $63.75 \mu\text{g/L}$  for *Chaetoceros* sp. It shows that *Chaetoceros* sp. used in this study was more sensitive than that of in the previous study.

Copper also influence the production of chlorophyll a (Figure 2). Chlorophyll-a decreased dramatically starting at  $44 \mu\text{g L}^{-1}$ . At low concentrations, copper is required by microalgae for transfer electron photosynthesis, cell metabolism, respiration, and enzyme co-factors that help the enzymes in certain reactions such in cells photosynthesis [10]. This is because an excessive concentration of Cu into microalgae may interfere the activity in chlorophyll, even though Cu is an essential metal. The Mg element is the important metal of the chlorophyll constituent. The excessive influx of Cu will reduce the amount of Mg intake and the possibility of the Mg element substitution in the chlorophyll. The  $\text{Mg}^{2+}$  is a weak acid and a weak metal compared to another cation. Therefore,  $\text{Mg}^{2+}$  can be replaced by  $\text{Cu}^{2+}$  which is a borderline element [11]. This causes the chlorophyll-a content decrease so that the productivity in photosynthesis will also be impaired. If photosynthesis is inhibited, it will reduce the rate of growth of microalgae [6,12].



**Figure 2.** Chlorophyll-a in *Chaetoceros* sp. after copper exposure within 96 hours.

At high concentration of Cu exposure, cell wall was deformed in the cell wall and setae disappeared (Figure 3). In addition, the high concentration of Cu cause luminescence of chlorophyll is reduced. The morphological changes of the cell wall can be explained by the process of Cu biosorption on the microalgae cell wall. The cell wall is the first cell barrier in heavy metal adsorption so it is a defensive mechanism that makes microalgae tolerates metal in its medium [13]. Biosorption process can occur in various ways. One of them is the physical adsorption with the formation of a bond with Van der Waals style between Cu and cell wall. Adsorption processes include the exchange of ions such as  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Si}^{4+}$  on the cell wall replaced by  $\text{Cu}^{2+}$  ions [14]. Increase in cell size at the higher copper concentration ( $140 \mu\text{g L}^{-1}$ ) in the present study may be due to inhibition of cell division and accumulation of photosynthesis products inside the cell [15].



**Figure 3.** Microscopic photographs (400x magnification) of cell morphology of *Chaetoceros* sp. in control (a) and in the copper concentration of 140  $\mu\text{g/L}$  (b) after 96-h. Setae was disappeared in copper treatment.

#### 4. Conclusions

Copper has inhibited 50% growth of phytoplankton *Chaetoceros* sp. in 30.25  $\mu\text{g L}^{-1}$ . Copper does not only inhibit the growth of microalgae, but also decrease the content of chlorophyll-a. Copper in high concentration also influences deformation of the cell wall and makes microalgae loses their setae.

#### Acknowledgments

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