Copper (Cu) and Cadmium (Cd) toxicity on growth, chlorophyll-a and carotenoid content of phytoplankton *Nitzschia* sp

To cite this article: D Hindarti and A.W. Larasati 2019 *IOP Conf. Ser.: Earth Environ. Sci.* 236 012053

View the article online for updates and enhancements.
Copper (Cu) and Cadmium (Cd) toxicity on growth, chlorophyll-a and carotenoid content of phytoplankton Nitzschia sp

D Hindartij, Larasati, A.W.2

1Research Center for Ocenaography, Indonesian Institute of Sciences, Jl. Pasir Putih I, Ancol Timur, Jakarta 14430.
2Universitas Gadjah Mada, Bulaksumur, Yogyakarta 55281

*Corresponding author: hindartidwi@gmail.com

Abstract. This research aimed to study the toxicity of copper (Cu) and cadmium (Cd) on growth, chlorophyll-a and carotenoid content of phytoplankton Nitzschia sp, and to study the IC50-96 h value on growth and chlorophyll-a content, LOEC, and NOEC. This research was done in Research Center of Oceanography, Indonesian Institute of Sciences through four steps: phytoplankton cultivation, definitive test, cell density counting, and chlorophyll-a and carotenoid test. Result showed that increasing heavy metals concentration lead to the decreasing of cell density and intracellular pigment content of Nitzschia sp. Based on the research, the IC50-96 h value of copper (Cu) and cadmium (Cd) on growth was 0.268 mgCu/L and 0.159 mgCd/L. The IC50-96 h value of copper (Cu) and cadmium (Cd) on chlorophyll-a content was 0.274 mgCu/L and 0.150 mgCd/L. LOEC to Nitzschia sp. was 0.18 mgCu/L and 0.18 mgCd/L, while NOEC to Nitzschia sp. was 0.1 mgCu/L and <0.18 mgCd/L. Copper and cadmium toxicity significantly affected the carotenoid content of toxicant concentration of 0.1 mgCu/L and 0.18 mgCd/L.

1. Introduction

Improvement of industrial development in Indonesia rapidly occurred along with the increase in science and technology. Nevertheless, the increase in the industrial field has a negative impact on environmental health by environmental pollution. Pollution is the existence of hazardous substance into the environment and changes the use of the environment itself [1]. Marine ecosystem has the highest levels of pollution because all the waste released from water bodies flows into the oceans and potentially alters the character of the aquatic communities. Those waste discharged into the aquatic environment contains toxic substances such as hydrocarbons and heavy metals which are toxic at low concentrations [2]. Essential metals such as iron (Fe), zinc (Zn), copper (Cu), cobalt (Co), have an important roles in the metabolic system as trace metals essentials at low concentrations. However, at higher concentrations, the heavy metals are capable of causing toxicity and disrupting growth [3,4]. Copper (Cu) is required by the biological system as a cofactor of some oxidative stress inhibiting enzymes such as catalase, peroxidase, cytochrome c oxidase, monoamine oxidase, and dopamine β-monooxigenase. Specifically, copper acts as a prosthetic group in the antioxidant enzyme superoxide dismutase in chlorophyll [5] however, copper ion is the most toxic heavy metal to aquatic organisms. Copper is toxic to phytoplankton in the range of 5 to 100 μg/l [3].

Cadmium is the most dangerous heavy metal in the environment [6]. It causes ROS (reactive oxygen species) and oxidative stress which affect its toxicity and carcinogenicity. Cadmium along
with arsenic (As), mercury (Hg), chromium (Cr), and lead (Pb) are referred as systemic toxicant that cause various damage at exposure in low concentration [4]. In microalgae and macroalgae, cadmium is toxic at concentrations of 22.8 to 860 µg/L [7].

Accumulation of heavy metals in aquatic organisms such as phytoplankton does not always produce toxic effects, but potentially causing biomagnification at the food chain, posing a threat to higher organisms [6]. Nevertheless, the decline in phytoplankton populations may be due to deterioration in environmental quality because of the high concentrations of heavy metals in seawater [8].

*Nitzschia* sp. is a benthic phytoplankton which is potential as a test parameter because of its cost-effectiveness and found easily because of its wide distribution. Furthermore, phytoplankton is highly flexible in various study, resistant, selective in heavy metals, and capable of reducing metal concentrations [6].

In this research, IC$_{50}$ (Inhibition Concentration), LOEC and NOEC values are calculated to detect any pollution and to understand the effect of toxicant to aquatic organisms [9]. Endpoint of this toxicity test include growth (cell density), chlorophyll-$a$ content, and carotenoid of *Nitzschia* sp.

### 2. Material and methods

#### 2.1. Phytoplankton cultivation

All of glass wares used in this research were washed sterilized using standard cleaning procedures described in American Standard Testing Materials [10]. All glass wares were soaked in 10% HNO$_3$ overnight and then rinsed with distilled water and acetone in 3 repetitions each. The washed glass wares were then sterilized using autoclave and dried in the oven. *Nitzschia* sp. obtained from the Laboratory of Mariculture, Research Center for Oceanography, Indonesian Institute of Sciences was used as test biota. *Nitzschia* sp. is a benthic diatom with silicate coated cell. To support its growth, *Nitzschia* sp. is cultured in an F2 medium rich silicate as silicate (Si) is a limiting factor for diatom growth. The phytoplankton was cultured to increase cell number in Erlenmeyer flasks containing enriched seawater with F2 with EDTA (*Ethylene Diamine Tetraacetic Acid* chelating agents) culture medium in room temperature (27°C) with oxygen aeration and covered by aluminum foil. The cultivation temperature was maintained at 27°C, and the flasks were illuminated in a continuous light (11080 lux). F2 culture medium, one of the commonly used medium for algae culture, was prepared by adding 1 mL of NaNO$_3$, NaSiO$_3$, NaH$_2$PO$_4$, 1 mL of trace metals solution; and 1 mL of vitamins into 1 L filtered (Sartorius cellulose nitrate filter, 0.45 µm pore size, 47 mm in diameter) and sterilized (autoclaved for 15 minutes at 15 psi) seawater.

Composition of F2 culture medium is presented in Table 1. The density of phytoplankton was calculated every 24 hours using Haemocytometer (*Improved Neubauer Assistant Germany*) until the cell density decrease towards their death phase. The phytoplankton culture was prepared for growth curve observation of *Nitzschia* sp. to obtain exponential phase that can be used for the growth inhibition tests of algae exposed to heavy metals [11]. The cells in mid-exponential growth phase of the phytoplankton took place after about four days culture (Fig. 1). In this phase, phytoplankton has well-adapted to its new environment and grow optimally. One of requirement for phytoplankton as test organism in toxicity testing is rapid growth [8].

| Nutrient solution Materials | Per litre | Trace element solution Materials | Per litre | Vitamin solution Materials | Per litre |
|-----------------------------|----------|---------------------------------|----------|---------------------------|----------|
| NaNO$_3$                    | 75.0 g   | ZnSO$_4$.7H$_2$O                 | 22.0 g   | Thiamine                  | (vit. B1)| 200 mg |
| Na$_2$HPO$_4$.H$_2$O        | 5.0 g    | CoCl$_2$.6H$_2$O                 | 10.0 g   | Cyanobalamin (vit. B12)   | 1 g     |
| NaSiO$_3$.9H$_2$O           | 30 g     | MnCl$_2$.4H$_2$O                 | 180 g    | Biotin                    | 1 g     |
|                             |          | CuSO$_4$.5H$_2$O                 | 9.8 g    |                           |         |
The 1st International Conference on Fisheries and Marine Science
IOP Conf. Series: Earth and Environmental Science 236 (2019) 012053
doi:10.1088/1755-1315/236/1/012053

FeCl$_3$.6H$_2$O 3.15 g
Na$_2$EDTA.2H$_2$O 4.36 g
Na$_2$MoO$_4$.2H$_2$O 6.3 g

Figure 1. Growth curve of Nitzshia sp.

2.2. Growth inhibition test
Growth inhibition tests were performed followed the standard procedures [10] and [14]. The test was carried out by inoculating the 4 days old growing phase cultures of Nitzchia sp. in 100 mL F2 media without EDTA with an initial density of 1 x 10$^4$ cells/mL in 250 mL sterilized Erlenmeyer flasks. Stock solution of cadmium and copper were prepared in distilled water respectively using their metallic salt of cadmium chloride dehydrate (CdCl$_2$.H$_2$O, Merck) and copper sulfate pentahydrate (CuSO$_4$.5H$_2$O, Merck). The 96-h toxicity test was performed in triplicate experiments using different nominal concentrations of Cd (0.18, 0.32, 0.56, 1.00, 1.80, and 3.20 mg/L) and of Cu (0.018, 0.032, 0.056, 0.100, 0.180, and 0.320 mg/L). All flasks were covered with aluminum foil to prevent any contamination. All flasks were placed in randomized position in the incubation chamber with continuous illumination (cool white fluorescent lighting, with the light intensity of 11000 lux) and shook twice daily to prevent precipitation. Test conditions were recorded for Cd (temperature 26.07-26.33°C, pH 8.38-8.44, salinity 30.1-30.4 ppt, dissolved oxygen 5.24-5.59 mg/L), and for Cu (temperature 26.05-26.39°C, pH 8.24-8.49, salinity 30.0-30.5 ppt, and dissolved oxygen 6.06-6.74 mg/L). Tests were terminated after 96 hours exposure by transferring 0.9 mL subsample from each Erlenmeyer flask into the labeled glass vial and preserved with 0.1 mL Lugol’s solution. Cell density was calculated Haemocytometer under high power standard bright-field microscope (Nikon Eclipse E600).

2.3. Analysis of chlorophyll-a and carotenoid content of Nitzschia sp.
The 96-hour phytoplankton culture from each treatments and controls were filtered through 0.45 μm Sartorius filter paper (Sartorius cellulose nitrate filter, 0.45 μm pore size, 47 mm in diameter) for chlorophyll-a and carotenoid analysis. The filter paper containing phytoplankton was then folded and wrapped in aluminum foil to prevent light penetration and labeled before being stored in the refrigerator until analysis was performed.

Intracellular pigment analysis was done by extracting filter paper containing phytoplankton with 10 ml of 80% acetone. The samples were then centrifuged in 3000 rpm for 20 minutes to segregate filtrates of the pigments. Absorbance test was carried out by UV-Vis spectrophotometer at a wavelength of 450 nm, 645 nm, and 663 nm to determine the chlorophyll-a and carotenoid concentration of phytoplankton [15] (Cochlan and Hendorn 2012).

From the extraction, total carotenoids and chlorophyll levels were determined by UV Visible Spectroscopy split beam spectrophotometer of samples in N,N-dimethylformamide
(DMI") using the equation proposed by [16]. The concentration of total carotenoid was calculated using following equation:

\[
\text{Chlorophyll a (Ag mL\textsuperscript{-1})} = 11.24 \times A6616 - 2.04 \times A6448 \\
\text{Chlorophyll b (Ag mL\textsuperscript{-1})} = 20.13 \times A6448 - 4.19 \times A6616 \\
\text{Total carotenoids (Ag mL\textsuperscript{-1})} = (1000 \times A470 - 1.90 \times \text{Chl a} - 63.14 \times \text{Chl b}/214)
\]

2.4. Data Analysis

Algal cell densities for each flask were measured at the end of the tests and calculated as cell numbers (algae cells ml\textsuperscript{-1}). The percent growth inhibition (I) is calculated using the equation below.

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

Where C was an average cell densities in the control and T was an average cell densities in the treatments. The ICPIN software application version 2.0 [17] was used to estimate 96-h IC\textsubscript{50} values with 95% confidence intervals (CI), with employing the responses and measured toxicant concentration data from all concentrations. Responses were considered significantly different when their 95% CI did not overlap [18]. One-way ANOVA was conducted to determine significance effects of Cd, Cu and controls on cell densities, chlorophyll-a and carotenoid content of the phytoplankton, respectively.

3. Result and discussion

3.1. Toxicity of Copper (Cu) and Cadmium (Cd) to growth of Nitzschia sp.

After 96 hours exposure to the metals, there is decrease in density of Nitzschia sp. and an increase in percent inhibition in each treatment compared to controls. Figure 2 shows the density reduction did not occur consistently after 96 hours exposure of Cu. The concentration of 0.032 mg/L was presumably to causes stimulation of phytoplankton cell growth according to the function of copper as a component of essential trace metals. In low concentrations, copper is not toxic but will actually spur cell growth by becoming a cofactor for various growth enzymes. Copper is a component of plastocyanin in the electron transport chain in photosynthetic reactions [18]. At a concentration of 0.018 mg/L, cell density was lower than the control and treatment of 0.032 mg/L with the assumption that at this concentration, deficiencies of copper micronutrient occurs and affect the cell growth becomes less optimal and tends to slow down. It is assumed that copper toxicity has not occurred yet because cell density is still increasing at a higher concentration of 0.032 mg/L. At concentrations of 0.056 mg/L to 0.1 mg/L, there was a constant decrease in density so that it can be said that the toxic effects of copper have shown a response to phytoplankton growth Nitzschia sp. However, at a concentration of 0.18 mg/L, there is an increase in cell density compared to the previous concentration. It is assumed that copper precipitation occurs in seawater so that homogeneous shaking when preparing for toxicity tests greatly affects the results of observations.
Figure 2. % inhibition of copper (Cu) and its toxicity to phytoplankton Nitzschia sp. after 96 hours of exposure

The highest test concentration of 0.32 mg/L showed a very high growth inhibition compared to the control which is 69.03% with cell density of $8.75 \times 10^4$ cells/mL. The percent inhibition graph states that the IC$_{50}$-96 hour value of copper (Cu) for phytoplankton growth is 0.268 mg/L. According to [8], copper (Cu) toxicity in phytoplankton causes disruption of cell wall function by reducing the concentration of K$^+$ ions in the cell and disrupt electron transport. The excess of Cu$^{2+}$ will accumulate on the cell wall, absorbed into the cell and affect the enzyme by binding to the sulfhydryl group (-SH). The enzyme disrupted and affect the reproductive ability.

![Figure 2](image_url)

Figure 3. % inhibition of Cd (Cadmium) and its toxicity to phytoplankton Nitzschia sp. after 96 hours of testing

The cell density of control treatment was $20.92 \times 10^4$ cells/mL. The decrease in cell density was significant and consistent at each concentration that is $9.08 \times 10^4$ cells/mL in the treatment of 0.18 mg/L, $6.83 \times 10^4$ cells/mL in the treatment of 0.32 mg/L, $6.33 \times 10^4$ cells/mL in the treatment of 0.56 mg/L, $4.00 \times 10^4$ cells/mL in the treatment of 1.00 mg/L, $2.33 \times 10^4$ cells/mL in the treatment of 1.80 mg/L, and $0.67 \times 10^4$ cells/mL in the treatment of 3.20 mg/L. Percent inhibition has reached > 50% in the treatment of 0.18 mg/L which is 56.57%. Based on ICPIN statistical data analysis, 96-hour IC$_{50}$ value of cadmium (Cd) to phytoplankton growth is 0.159 mg/L. From previous studies by [19], [9], and [8] show the consistency of cadmium toxicity test results. Therefore, cadmium metal is used as a reference toxicant for each toxicity test. Reference toxicant serves as a positive control to evaluate the sensitivity of the test biota with the toxic requirement to provide a consistent response to various test biota in various concentrations. The positive control shown by an increase in cadmium concentration will reduce the density of phytoplankton cells [19].

Cadmium causes morphological disruption especially in the process of cell division. In addition, cadmium affects protein synthesis and cell organelles such as mitochondria. Mitochondrial swelling occurs in various types of diatoms. In a previous study, the toxic effect of cadmium caused Euglena gracilis lost its motility and Thalassiosira fluviatilis and T. aestivalis lost the ability to absorb nitrate and disrupt cell metabolism by inducing cell elongation [6]. Cadmium can be adsorbed on the cell wall thus disrupting nutrient transport. Cadmium can also enter the cell and replace the essential divalent metal ions in enzymes [6]. Enzymes for metabolic processes become disrupted through blocking and reduction of thiol groups in proteins. Thiol group is a group that play roles in the formation of various antioxidant enzymes, one of them is glutathione and metallothionein [8]. The presence of these metal ions interferes the oxidation reduction reaction in the cell. Free radicals in cells or ROS (reactive oxygen species) which can be reduced by antioxidant enzymes, are not reduced because the
antioxidant enzyme binds Cd\(^{2+}\) ions. The excessive amounts of Cd\(^{2+}\) ions in the cell cytoplasm of *Nitzschia* sp. causing disruption of cell homeostasis [20].

In general, heavy metals have the ability to inhibit cell growth through two stages, passive absorption and active absorption. Passive absorption occurs by the interaction of heavy metals and cell walls while active absorption occurs by transporting heavy metals through the cell membrane into the cytoplasm. Passive absorption occurs when the concentration of extracellular metal is higher than its intracellular concentration, whereas active absorption requires energy from the cell to absorb extracellular metal ions. Heavy metal ions then replace the essential enzyme cofactors and damage the cell organelles causing the cell structure lose carbohydrates, decrease the vacuoles, and the most significant effect is interfere the chloroplasts [19]. Heavy metal ions that interact with cell components such as DNA and nucleic proteins will cause DNA damage. DNA damage affects the presence of carcinogens in cells and causes apoptosis of cell cycle. In previous studies, it was indicated that cadmium influences signal transduction, induction of inositol polyphosphate formation, and closes protein channels. At low concentrations (1-100 μM), cadmium binds proteins, reduces DNA repair ability, activates protein degradation, and induces several genes that encode metallothionein, heme-oxygenase, glutathione transferase, and DNA polymerase [4]. The toxicity of heavy metals can be reduced through several mechanisms such as the production of heavy metals and proteins chelating agent (metallothionein and conjugate phytochelatins), the release of heavy metals from cells through "ion-selective metal transporters" and excretion or compartmentalization. Phytoplankton tolerance to heavy metals is very dependent on the defense response to oxidative damage, binding ability of chelating compounds, heavy metal active efflux by ATPase pumps, and decreasing absorption of heavy metals themselves. Previous research has shown that metal-binding proteins, metallothionein and cysteine-rich polypeptides, play an important role as metal-binding ligands. In addition, phytoplankton can produce proline (Pro). Proline reduces heavy metal stress through free radical detoxification. The mechanism that occurs is that Pro reduces free radical damage such as physical collisions from singlet oxygen and chemical reactions with hydroxyl radicals [20].

Growth inhibition in phytoplankton is related to the number of heavy metal ions that are bound to the cell surface, the number of intracellular heavy metal ions, and the natural chemical properties of these heavy metal ions. Heavy metal ions cause depolarization of plasma membrane and cytoplasmic acidification. Membrane damage is the main effect caused by heavy metal ions which affect cell homeostasis. In the growth medium, the presence of heavy metal ions induce peroxide activity which is responsible for the degradation of IAA enzymes [20].

3.2. Chlorophyll-a and carotenoid content of *Nitzschia* sp.
Based on absorbance test, copper (Cu) and cadmium (Cd) has toxic effect and causes the decrease in chlorophyll-a and carotenoids content of *Nitzschia* sp. in each treatment compared with controls.

![Figure 4. Chlorophyll-a and carotenoid content of *Nitzschia* sp. after 96 hours of Cu exposure](image-url)
It can be said that the number of measured cells in the calculation of cell density is positively correlated to the content of chlorophyll-\(a\). Increased concentration of chlorophyll-\(a\) at a concentration of 0.032 mg/L showed the role of copper as an essential micronutrient in growth, formation of thylakoid, and as a cofactor of antioxidant enzymes. Copper (Cu) toxicity was shown at concentrations of 0.056 mg/L, 0.1 mg/L, and 0.32 mg/L. At higher toxicity concentrations, chlorophyll-\(a\) content decrease because \(\text{Cu}^{2+}\) inhibits the synthesis of d-aminolevulinic acid and protochlorophyll reductase [21]. De Filippis et al. (1981) in [21] explained that reduction of chlorophyll-\(a\) content is the main effect of heavy metals presence in cells. The growth of \textit{Nostoc calcicola} was severely inhibited due to lack of photosynthetic pigments (phycocyanin > chlorophyll-\(a\) > carotenoids). It can be said that high metal concentrations can change the permeability of the plasma membrane and damage the function of the cell membrane. Wilde et al. (2006) and Wong et al. (2004) in [20] say that copper induction causes changes in the structure of the thylakoid membrane \textit{Chlorella} sp. and inhibits its photosynthesis process.

In carotenoid measurements, the increase only occurred at a concentration of 0.032 mg/L and continued to decrease consistently to a concentration of 3.2 mg/L. Based on the ANOVA test, it was known that copper affected carotenoid biosynthesis at a concentration of 0.1 mg/L. This shows that carotenoids as antioxidants is not able to inhibit the interference of heavy metal ions. Carotenoid content have decreased due to not being able to inhibit the presence of ROS (reactive oxygen species) that damage photosynthetic pigments. In previous studies, it was found that high concentrations of \(\text{Cu}^{2+}\) caused a reduction in photosynthetic pigments from \textit{Chlorella} sp. \(\text{Cu}^{2+}\) transport occurs through binding of ions with ligands on the cell surface. The ion-ligand complex then enters through the plasma membrane and forms a complex with a stronger ligand inside the cell itself [18].

**Figure 5.** Chlorophyll-\(a\) and carotenoid content of \textit{Nitzschia} sp. after 96 hours of Cd exposure

Based on figure 5, it is known that the excessive cadmium content inhibit intrathylakoid development, thylakoid degradation, and increase the number and size of polyphosphates and cyanophycin granules to cause lysis cells. The presence of heavy metals in the growth medium causes decrease in chlorophyll-\(a\) content due to degradation of the thylakoid membrane. The process of capturing light as a source of photosynthetic energy will disrupted, causing interference with the formation of ATP and NADPH so that the cell does not have the energy to run its metabolism. The absence of energy formed from photosynthesis causes cells unable to synthesize essential organic compounds in the body and cause cell death [19].

In ionized cadmium, \(\text{Cd}^{2+}\) disrupts the function of chloroplasts by damaging their photosynthetic pigments and inhibiting the photosynthetic activity of phytoplankton. Without the photosynthesis process, the energy for cell metabolism cannot be met. Disruption of cell metabolic processes causes growth inhibition and eventually causes cell death. Heavy metal ions can interfere with the metabolic process through competition with active protein groups, activating enzymes and other reactive groups.
that cause growth inhibition to zero. Carotenoid biosynthesis can be affected by heavy metal ions. The presence of heavy metal stress induce carotenoid production, but excessive exposure to heavy metals will reduce the content of carotenoids themselves [20]. Reduction of photosynthetic pigments both chlorophyll-α and carotenoids indirectly influences the photosynthetic ability of *Nitzschia* sp.

From this study, it is known that the IC₅₀-96 values of copper (Cu) and cadmium (Cd) to growth are 0.268 mgCu/L and 0.159 mgCd/L respectively. Whereas IC₅₀-96 hours of copper (Cu) and cadmium (Cd) for chlorophyll-α content were 0.274 mgCu/L and 0.150 mgCd/L respectively. Based on the IC50-96 hour value on growth and on chlorophyll-α content, it can be concluded that cadmium metal is more toxic than copper metal. IC₅₀-96 hour analysis of growth shows the same trend as IC₅₀-96 hour analysis of chlorophyll-α. However, from this study, the recommended IC₅₀-96 hour analysis is a test of chlorophyll-α with the consideration that the analysis with chlorophyll-α shows better results because the measurements made are actually from test biota that still able to do their metabolism well by the presence of chlorophyll-α. In IC₅₀-96 hour analysis of growth, the overall biomass of cells will be counted both dead and alive so that they can show biased calculation results.

The lowest concentration that significantly reduce population growth of *Nitzschia* sp. (LOEC) for copper (Cu) was 0.18 mg/L and cadmium (Cd) was 0.18 mg/L. The highest toxicity concentration that has no significant effect on population growth of *Nitzschia* sp. (NOEC) for copper (Cu) and cadmium (Cd) were 0.1 mgCu/L and < 0.18 mgCd/L respectively. From these results, it is known that *Nitzschia* sp. has a higher sensitivity to cadmium metal than copper. From IC₅₀-96 hour value on growth, cadmium toxicity is 1.7 times stronger to inhibit phytoplankton growth compared to copper; and cadmium 1.8 times stronger in inhibiting the growth of phytoplankton compared to copper based on IC₅₀-96 hour values on chlorophyll-α content.

In this study, IC₅₀-96 hour values of Cu for growth and chlorophyll-α levels were higher than the LOEC and NOEC values. However, the treatment with cadmium, IC₅₀-96 hour values on growth and chlorophyll-α content obtained were lower than the LOEC and NOEC values. This is caused by the objectivity of the two values. The IC₅₀-96 hour value is obtained based on the survival ability of the test biota on the toxic exposure given while the NOEC and LOEC values were obtained based on the concentration range made by the researcher. Therefore, a lower IC₅₀-96 hour value occurs because the concentration range of the toxicity test made is too high. In addition, it is assumed that the test biota is more sensitive to the actual test (definitive test) so that the IC₅₀-96 hour value obtained is lower than the preliminary test (range finding test). From the research, it shows that copper (Cu) and cadmium (Cd) inhibit growth, chlorophyll-α, and carotenoid content of phytoplankton *Nitzschia* sp.

### 4. Conclusion

The research that has been done obtained results showed that increasing heavy metals concentration lead to the decreasing of cell density and intracellular pigment content of *Nitzschia* sp. Based on the research, the IC₅₀-96 h value of copper (Cu) and cadmium (Cd) on growth was 0.268 mgCu/L and 0.159 mgCd/L. The IC₅₀-96 h value of copper (Cu) and cadmium (Cd) on chlorophyll-α content was 0.274 mgCu/L and 0.150 mgCd/L. LOEC to *Nitzschia* sp. was 0.18 mgCu/L and 0.18 mgCd/L, while NOEC to *Nitzschia* sp. was 0.1 mgCu/L and <0.18 mgCd/L. Copper and cadmium toxicity significantly affected the carotenoid content of toxicant concentration of 0.1 mgCu/L and 0.18 mgCd/L.

### 5. References

[1] Briggs D 2003 *Environmental Pollution and the Global Burden of Disease* (British Medical Bulletin The British Council) p 1-24.

[2] Owa F D. 2013 *Mediterranean Journal of Social Sciences*. MCSER Publishing. Italy. 4 8

[3] Gautam R K, S K Sharma, S Mahiya and M C Chattopadhyaya. 2015. "*: Contamination of Heavy Metals in Aquatic Media: Transport, Toxicity, and Technologies for Remediation“, *Heavy Metals in Water: Presence, Removal, and Safety*. (UK. Sharma (Ed.). Royal Society of Chemistry) p 110
Acknowledgment

The authors would like to express their gratitude for all the Reviewers and Editors comments that helped in the manuscript improvement. This research was supported by Dr. rer. nat. Andhika Puspito Nugroho and Eston Matondang, A.Md. (dec.), and Laboratory of Mariculture and Laboratory of Marine Chemistry and Ecotoxicology – Analysis of Anorganic Chemistry, Research Center for Oceanography, Indonesian Institute of Sciences.

[4] Tchounwou P B, C G Yedjou, A K Patlolla and D J Sutton 2012. EXS 101: 133-164
[5] Kumar K S, H Dahms, J Lee H, Y Kim, W C Lee and K Shin 2014 Ecotoxicology and Environmental Safety 104 51-71
[6] Kaur I and A K Bhatnagar 2002 Algae-dependent Bioremediation of Hazardous Wastes in Biotransformation: Bioremediation Technology for Health and Environmental Protection. Elsevier, pp. 457-517
[7] United States Environmental Protection Agency 1993 Water Quality Criteria, pp. 47 and 83
[8] Suratno, R Puspitasari, T Purbonegoro and D Mansur 2015 Indonesian Journal of Chemistry. 2 172-178
[9] Desratriyanti R. 2009. Toxicity of cadmium (Cd) and copper (Cu) on the embryo-larval development of green mussle Perna viridis. Skripsi (Bogor Agriculture University. In Indonesian) p 201
[10] American Standards Test Materials. Standard Guide for Conducting Toxicity Tests with Microalgae Annual Book of ASTM Standards 2006. Section Eleven : Water and Environmental Technology. (Biological effects and Environmental Fate; Biotechnology) p 278–91
[11] Guillard R R L and Ryther, J H 1962 Journal Microbiology. 8: 229-239.
[12] Guillard R R.L 1975. Culture of phytoplankton for feeding marine invertebrates. In Smith W.L. and Chanley M.H (Eds.) (Culture of Marine Invertebrate Animals. Plenum Press, New York, USA) p 26-60
[13] Asean Canada Cooperative Programme on Marine Science Phase II. 1995. Draft Protocol for Subtidal Toxicity Tests Using Tropical Marine Organisms. Regional Workshop on Chronic Toxicity Testing (Burakha University, Institute of Marine Science) p 14-19
[14] Lichtenthaler H K and Buschmann C 2001 Chlorophylls and carotenoids: Measurements and characterization by UV-VIS Spectroscopy. In Current Protocols in Food Analytical Chemistry. John Wiley & Sons Inc. p. F4.3.1-F4.3.8
[15] Norberg King T 1993 A Linear Interpolation Method for Sub lethal Toxicity (The Inhibition Concentration (Icp) Approach) (version 2.0) p 1-30
[16] Chakilam, S. R. 2012. International Journal of Botany 8 (4) 192-197
[17] Setiawati M D, D Hindarti and R Kaswadji 2009 Testing Toxicity in Lead and Cadmium in Microalgae Chaetoceros gracilis. Proceedings of the 16th Tri-University International Joint Seminar and Symposium. (Mie University, Japan) p 302
[18] Arunakumara K K I U and Z Xuecheng 2008 Journal of Ocean University of China (Oceanic and Coastal Sea Research). 7 (1) 60-64
[19] Veerapandiyan N, T Lenin, P Sampathkumar, A A. Sundaram and S P Sangeetha. 2014 Journal of Science Inventions Today 3 (6): 725-736