The effect of density as *Skeletonema costatum* bioremediation agent of copper (Cu) heavy metal concentration

N A Pratama¹, B S Rahardja²,³ and L A Sari²

¹Aquaculture Study Program, Faculty of Fisheries and Marine University of Airlangga, Surabaya 60115 Campus C Airlangga University, Indonesia.
²Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine University of Airlangga, Surabaya 60115 Campus C Airlangga University, Indonesia.
³Corresponding author: bs_rahardja@yahoo.co.id

Abstract. Heavy metals copper (Cu) was microelements needed by organisms in small quantities. The type of plankton that could be used as a bioremediation agent because of its abundance and resistance in nature was *Skeletonema costatum*. This study aims to determine the effect of different density of *Skeletonema costatum* exposure Cu and Determine the effect of exposure to the growth of Skeletonema costatum. The design used in this research was an experimental method. This research using Completely Randomized Design (CRD) with three treatments and six replications. Treatment used was 5,000 cells/ml (A), 10,000 cells/ml (B), and 15,000 cells/ml (C) of cells density at 1 ppm Cu concentration. The results of this study prove that the density of *Skeletonema costatum* could affect Cu uptake levels with the absorption percentage of treatment A was 78.62b ± 10.40; the absorption percentage of treatment B was 88.13ab ± 7.49; the absorption percentage of treatment C was 91.96b ± 4.87. Culture media that contained 1 ppm Cu concentration could affect the growth and growth phase of Skeletonema costatum. *Skeletonema costatum* that exposed cells with Cu has higher growth than with Cu reviews those not exposed.

1. Introduction

Heavy metals copper (Cu) is an element that is required by the micro-organisms in small amounts. Cu can be absorbed in acute or chronic depending on the amount, timing of exposure, and the exposure. Plankton species, which can be utilized as a bioremediation agent because of the abundance and resilience in nature, is *Skeletonema costatum* [1].

*Skeletonema costatum* bioremediation process occurs through a mechanism of passive transport and active transport. Passive transport mechanism takes place in the cell wall through the process of adsorption[2], *Skeletonema costatum* cell walls contain a carbonyl functional group, amine, thiol, hydroxyl, phosphate, and carboxyl groups [3]. *Skeletonema costatum* functional groups that can bind with heavy metals are hydroxyl functional groups [4]. The functional groups on the cell walls are negatively charged, while the positively charged ions on Cu [5].

An active transport mechanism that has bound hydroxyl currently Cu absorption into the cytoplasm. Cu in the cytoplasm will be synthesized with fitokelatin, that synthesized from glutathione by fitokelatin synthase enzyme. Cu binding to fitokelatin will form a complex compound Cu. Cu complex compounds will accumulate into the vacuole before going through the process of cell metabolism [6] *Skeletonema costatum* indirectly also require small amounts of Cu [7], Cu through the process of cell metabolism can be used for the formation of chlorophyll, thereby increasing adaptability and growth *Skeletonema costatum* [8].
Skeletonema costatum metabolic processes using complex compounds of Cu can increase growth, but when the Cu accumulation in the cell is too high can lead to weak growth. Skeletonema costatum growth increased or inhibited can keep the concentration levels of Cu in the waters.

2. Methodology

2.1 Place and time of research
The study, entitled influence Skeletonema costatum density as a bioremediation agent to the heavy metal concentration levels of Cu carried out in the Laboratory of Anatomy and Aquaculture, Faculty of Fisheries and Marine Resources, Airlangga University and the Laboratory of Nutrition, School of Public Health, University of Airlangga. Skeletonema costatum samples obtained from the Center for Development of Brackish Water Aquaculture (BBPBAP), Jepara, Central Java. The research was conducted in January - April 2019.

2.2 Tools and materials
The tools used in this study are tube Erlenmeyer volume of 500 ml, measuring cups volume of 1000 ml, flask, 250 ml, glass jars culture volume of 700 ml, glass jars culture volume of 3000 ml sample bottles dark 300 ml, syringe, pipette, hose aeration, airstone, tanks of seawater, electric heater, magnetic stirrer, test tubes, test tube rack, Sedgewick Rafter Counting Cell, glass objects, atomic absorption spectroscopy (AAS), microscope, analytical balance, fluorescent light 40 watt, hand counters, refractometer, pH meter, thermometer, lux meter, and plankton net size 4 μm. Materials used in the research are the seeds of pure Skeletonema costatum, Cu powder, sea water, distilled water, sea salt, liquid detergent, chlorine, Na-thiosulfate, tissue paper, a liquid fertilizer Conwy (walne), scrap paper, and plastic wrap.

2.3 Research methods
The design used in this study is the experimental method. This study uses a completely randomized design (CRD) with research conducted using three kinds of treatments and six replications cell density of Skeletonema costatum. Treatment of Skeletonema costatum cell density used is 5000 (Treatment A), 10000 (Treatment B), and 15,000 (Treatment C) cells/ml at concentrations of 1 ppm Cu.

2.4 Disinfection equipment and materials
Disinfection is the process of disabling unwanted organisms in a device or material. Equipment disinfecting using liquid detergent and chlorine by soaking, washed, and rinsed with fresh water. The seawater is disinfected through two stages. Namely, the seawater is filtered using a plankton net size of 4 μm and drained reservoirs, then the seawater in the tank aerated and supplied with a dose of 60 ppm chlorine for 24-48 hours. Chlorine is useful as a disinfectant that can kill harmful microorganisms that still are in the water and stick to the walls and bottom of the seawater reservoirs. Chlorine takes about 24-48 hours to make the process of destruction of enzymes in microorganisms [9].

The seawater that has been disinfected for 24-48 hours using chlorine must be neutralized using Na-thiosulfate. Na-thiosulfate useful to neutralize and remove chlorine contained in seawater. Na-thiosulfate is given into the sea at a dose of 30 ppm. The seawater can be used 48 hours after the administration of Na-thiosulfate [10].

2.5 Preparation of liquid fertilizer conwy (Walne)
Fertilizer given in Skeletonema costatum is a type of liquid fertilizer Conwy (Walne) [11]. How liquid fertilizer Conwy (Walne) by using solvent 1000 ml of distilled water and chemical fertilizers by KNO3 composition of 100 ppm, 10 ppm NaH2PO4, Na2SiO3 10 ppm, 5 ppm EDTA, FeCl3 1 ppm, and 0,001 ppm of Vitamin B12. Chemical fertilizers are dissolved in a sequence of which has the highest concentration [11].
2.6 Preparation stock Skeletonema costatum

Skeletonema costatum culture is a pure culture seedling originating from BBPBAP Jepara. Making the stock Skeletonema costatum conducted using culture volume of 500 ml glass jar container sizes of 700 ml culture [12] [13] [14] [15] [16]. Fertilizer culture given on Skeletonema costatum is a type of liquid fertilizer Conwy (Walne). Conwy dose of liquid fertilizer (Walne) used was 0.5 ml / l. Environment and culture, which is expected in this study, is 2000-6000 lux light intensity [17], salinity 15-34 ppt [18], the degree of acidity (pH) 7-8 [11] and a temperature of 28-33 ° C [19]. The intensity of light using a 40-watt fluorescent lamp with a distance of 10 cm above the surface of the culture media and 24-hour photoperiod. The calculation of the volume of Skeletonema costatum seeds are put into the culture medium according to [20] can use the formula:

\[
\frac{V_1}{V_2} = \frac{N_2}{N_1} \rightarrow V_1 = \frac{N_2 \times V_2}{N_1}
\]

Information: 
V1 = Volume required (ml)  
N1 = Initial stock Skeletonema costatum density (cells / ml)  
V2 = Volume of water in the treatment of culture medium (ml)  
N2 = Density beginning of treatment (cells / ml)

2.7 Preparation of Cu stock solutions

Cu stock solution was prepared by diluting. Dilution is done by inserting CuSO₄ as much as 25 mg and then dissolved into 250 ml of distilled water in a flask and shaken by twisting clockwise, so we get the stock solution concentration of 100 ppm Cu. The volume taken to obtain a concentration of 1 ppm Cu in Cu 100 ppm stock solution was 2.5 ml.

3. Observations and data collection

3.1 Bioremediation calculation Skeletonema costatum against Cu Absorption

Skeletonema costatum bioremediation ability to Cu can be determined by comparing the concentration of Cu before treatment began, and after eight days, the bioremediation process is complete. Measurement of Concentration of Cu in the culture medium was conducted in the Laboratory of Nutrition, School of Public Health, University of Airlangga, Surabaya using AAS with ppm. The calculation of the efficiency of absorption according to [21] can use the formula:

\[
Eff = \frac{C_0 - C_1}{C_0} \times 100\%
\]

Information:  
Eff = The efficiency of absorption (%)  
C0 = Concentration before treatment Cu (ppm)  
C1 = Concentration of Cu after the bioremediation process (ppm)

3.2 Growth Calculation Skeletonema costatum

Skeletonema costatum growth calculation is done every day for eight days, starting every 09.00 pm. Calculation of Skeletonema costatum growth is done by sampling the culture medium 1 ml in each treatment, and repetition is then inserted into Sedgewick Rafter Counting Cell[22] [23] [24] [25]. Calculation of Skeletonema costatum growth of cells in 1 ml according to [26] may use the formula:
Information: 
- $N = \frac{C \times 1000 \text{ mm}^3}{L \times D \times W \times S}$

- $N$ = Cell density (cells / ml)
- $C$ = total organisms found by the visual field observed (cell)
- $L$ = Length of groove (mm)
- $D$ = High-groove (mm)
- $W$ = Width of groove (mm)
- $S$ = Amount calculated groove

### 3.3 Data analysis

The parameters were analyzed using analysis of variance (ANOVA) with an error rate of 5%. Duncan range test can be done if there is significant [27]. Statistical analysis was performed using the application Statistical Product and Service Solutions (SPSS) 17.0. *Skeletonema costatum* water quality data were analyzed descriptively.

### 4. Results and discussion

The results of the study proved that the decreased levels of Cu concentrations in the culture medium *Skeletonema costatum*, so *Skeletonema costatum* has qualified to become an agent for bioremediation.

The results of the study proved that the density of *Skeletonema costatum* could affect the absorption of Cu levels in the culture medium. The results mean total absorption treatment A is 0.7862 ppm with a mean percentage of absorption of 78.62%, the average total absorption treatment B is 0.8813 ppm with a mean percentage of absorption of 88.13%, the average total absorption treatment C is 0.9196 ppm the average percentage of absorption of 91.96%.

| Treatment | Concentrations of Cu (ppm) Day 0 | Concentrations of Cu (ppm) Day 8 | Total Absorption (ppm) | Absorption Percentage (%) ± Standard Deviation |
|-----------|----------------------------------|----------------------------------|------------------------|-----------------------------------------------|
| A         | 1.2138                           | .7862                            | 78.62b± 10.40          |                                               |
| B         | 1.1187                           | .8813                            | 88.13ab± 7.49          |                                               |
| C         | .0804                            | .9196                            | 91.96a± 4.87           |                                               |

The results of the study proved that the culture medium containing 1 ppm Cu can affect the growth of *Skeletonema costatum*. *Skeletonema costatum* peak growth occurred on the 8th day. The results mean peak growth treatment A is 586.52 $\times 10^3$ cells/ml, mean peak growth treatment B was 942.92 $\times 10^3$ cells / ml., and the mean peak growth treatment C was 1331.83 $\times 10^3$ cells/ml. *Skeletonema costatum* growth without exposure to heavy metals based on the preliminary study indicated that the peak growth of *Skeletonema costatum* occurred on day 3 and 4 with the peak growth density treatment 5,000 cells/ml is 327.33 $\times 10^3$ cells/ml, the growth peak density of 10,000 cells treated / ml is 334.67 $\times 10^3$ cells/ml, the growth peak density treatment of 15,000 cells/ml is 902$\times 10^3$ cells/ml. *Skeletonema costatum* growth without exposure to heavy metals based study [17] showed that the peak growth of *Skeletonema costatum* occurred on the 3rd day of treatment with growth peak density of 5,000 cells/ml is 146.5$\times 10^3$ cells/ml, the growth peak density of 10,000 cells treated / ml is 184 $\times 10^3$ cells/ml, the growth peak density treatment of 15,000 cells/ml is 340$\times 10^3$ cells/ml. *Skeletonema costatum* exposed Cu has a higher number of cells than those not exposed to Cu, but the peak growth occurred for longer. A higher number of cells due to *Skeletonema costatum* also requires Cu in small quantities for metabolic processes [7].
Table 2. Average growth of *Skeletonema costatum* in Research

| Days to- | Average *Skeletonema costatum* cells (10^3 cells / ml) ± standard deviation |
|---------|---------------------------------------------------------------|
| 0       | A 5,000c ± 0.00 B 10,000b ± 0.00 C 15,000a ± 0.00          |
| 1       | 5,250b ± 0.97 A 9,38 ± 1.54 B 10,25a ± 1.74                |
| 2       | 19,21b ± 1.93 28,65a ± 6.32 B 34,13a ± 8.95              |
| 3       | 31,38b ± 4.45 46,50ab ± 8.97 A 58,55a ± 32.45             |
| 4       | 57,25b ± 8.16 151,71a ± 34.48 A 185,48a ± 58.40          |
| 5       | 113,95b ± 31.20 299,70a ± 102.07 A 321,68a ± 109.12      |
| 6       | 256,95b ± 70.61 482,95a ± 173.72 A 539,33a ± 122.27      |
| 7       | 555,33b ± 84.61 774,00a ± 134.27 A 741,67a ± 126.77      |
| 8       | 586,52c ± 65.17 942,92b ± 156.81 A 1331,83a ± 398.48     |

The results of the study proved that the culture medium containing 1 ppm Cu phase could affect the growth of Skeletonema costatum. The growth phase on the research showed that the resting phase occurs in 0-3 days of maintenance, exponential phase occurs at 3-7 days of maintenance, and the stationary phase and the phase of the deaths occurred in 7 days or more maintenance. *Skeletonema costatum* growth phase without exposure to heavy metals based on the preliminary study indicated that the resting phase occurs in 0-2 days of maintenance, exponential phase occurs at 2-4 days of maintenance, stationary phase occurs in 4-5 days, and the death phase occurred in 5 days or more maintenance. *Skeletonema costatum* growth phase without exposure to heavy metals based study [17] showed that the resting phase occurs in 0-2 days of maintenance, exponential phase occurs in 2-3 days maintenance, stationary phase occurs in 3-4 days, and the death phase occurs in 4 days or more maintenance. *Skeletonema costatum* growth phase in a longer exposure of Cu occurs because *Skeletonema costatum* is still in the stage of adaptation to the environment that was not previously exposed by Cu.

The results mean and the mean percentage growth is the lowest absorption treatment A, while the results mean and the mean percentage growth is the highest absorption treatment C. Cu concentration levels that are too high can cause toxicity in the cells because too much copper can accumulate in the cell and become an inhibitor. Cu, which becomes inhibitors, can slow the rate of cell metabolism, growth low, to cause death. Cu is too high accumulation in cells may occur because of the density of *Skeletonema costatum* is too low over the concentration of Cu in an environment that is too high [28].

Water quality also affects the levels of heavy metal toxicity and cell state Skeletonema costatum. Results of water quality measurements *Skeletonema costatum* culture medium throughout the study
shows the range of 2172-2240 lux light intensity. The less appropriate light intensity can cause cracking process *Skeletonema costatum* energy in cells is inhibited. The intensity of light that is too low can cause the light reaction of photosynthesis runs less than optimal due to changes in water and NADP into NADPH requires oxygen and light to ionize molecules in Photosystem II, so that the electrons are in the system II will be released and will be converted into ATP. The light intensity is too high can lead to the closing of stomata on the cell because it can damage the complex compounds in cells [29].

*Skeletonema costatum* salinity culture medium during the study demonstrates the range of 21-28 ppt. Every day the culture medium salinity increased due to the mineral content in seawater do not evaporate and will continue to accumulate in the culture medium. Higher salinity may lead to a process of binding heavy metals on the mineral content of seawater so that the lower the toxicity of heavy metals. Heavy metal toxicity will be low due to heavy metals can bind to the mineral content in ea water, so that decreased levels of heavy metal concentrations in the culture medium could be due to the salinity of the culture medium of the higher [30].

*Skeletonema costatum* pH of the culture medium during the study demonstrates the range of 6.9 to 7.5. The lower pH can cause heavy metals more easily soluble in water because the water is acidic and bind to molecules on the metal so that the toxicity of heavy metals may increase. The pH of the culture medium tends to be in the range of neutral study so that the pH does not affect the toxicity of Cu in the culture medium.

*Skeletonema costatum* culture media temperature during the study shows the range of 29-34 °C. Temperatures higher than optimal levels of *Skeletonema costatum* can speed up your metabolism so that growth will be higher and the absorption of heavy metals can run faster, but if the temperature is too high that can not be tolerated by *Skeletonema costatum* it can cause death because it can damage cells. Temperatures that are too high can result in cell lysis because of the destruction of the protein molecule that the enzyme forming process may be interrupted.

5. Conclusion

*Skeletonema costatum* density can affect the absorption of Cu levels in the culture medium, the absorption percentage results of treatment A is 78,62 ± 10.40; the percentage of absorption treatment B is 88,13 ± 7.49; absorption percentage 91,96 treatment C is ± 4.87. The culture medium containing 1 ppm Cu concentrations can affect growth and growth phase *Skeletonema costatum*. *Skeletonema costatum* exposed Cu has a higher number of cells than those not exposed to Cu, but the peak growth occurred for longer.

6. References

[1] Maulana A, Supartono, and S Mursiti 2017 *J Chem Sci*. 6 256-261.
[2] Nassiri J L Mansot, J We'ry, T Ginsburger-Vogel and J C Amiard 1997 *Arch. Environ. Contam. Toxicol* 33 147-155.
[3] Suhendrayatna 2001 *Sinergy Forum-PPI Tokyo Institute of Tech*. 1-9
[4] Soedarti T, L R Maryono, S Hariyanto 2016 *icsbe-Fourth International Conference on Sustainable Built Environment* 263-270.
[5] Devinta 2013 *J Sci Arts Points* 2 337-352.
[6] Kok T 2013 *National Seminar on Science and Technology V, Research University of Lampung* 497-507.
[7] Arifin Z 2007 *Wartazoa - Indonesian bulletin of Animal and Veterinary Sciences* 17 93-99.
[8] Fauziah F, R Wulansari, E Rezamela 2018 *Agrikultura Journal* 29 26-34.
[9] Hasan A 2006 *Journal of Environmental Technology* 7 90-96.
[10] Leonard R, E D Masithah, W Tjahjaningsih 2017 *Marine Technology for Sustainable Development* 7 12-20.
[11] Armanda D T 2013 *Biome* 2 49-63.
[12] Setyawati F, W H Satyantini, M Arief, Kismiyati, Pujiaustuti 2018 *Journal of Aquaculture and Fish Health* 7 50-56.
[13] Daughter A D A, W Tjahjaningsih 2018 *Journal of Aquaculture and Fish Health* 7 111-117.
[14] Mubarak U S, L Sulmartiwi, D T R Tias *Scientific Journal of Fisheries and Marine* 1 67-72.
[15] Arsad S, C Stavrakakis, V Turpin, P Rossa, Y Risjani, L A Sari, F S Prasetya, J L Mouget 2019 *IOP Conference Series: Earth and Environmental Science* 236 012-044.
[16] Sari L A, E D Masithah, M A Alamsjah 2018 *Journal of Fisheries and Marine Research* 2 9-14.
[17] Rahmanianda A 2015 *Bioremediation of Heavy Metal Cadmium (Cd) by Skeletonema sp.* *Essay* (Surabaya: Airlangga University) p 19-47.
[18] Supriyantini E 2013 *Bulletin Oceanographic Marina* 2 51-57.
[19] Wisudiyawati D 2014 *The Comparative Study of Ability Skeletonema sp. and Chaetoceros sp. as a Bioremediation Agent (Phy-accumulation) to Heavy Metal Lead (Pb)* (Surabaya: Airlangga University) p 6-28.
[20] Umairina M R, U S Mubarak, E D Masithah 2012 *Effect of Leaf Fertilizer Concentration White Turi (Sesbania grandiflora) against the population of Chlorella sp* (Surabaya: Airlangga University) p 1-9.
[21] Gupta R P, A S Khan, R K Sakena, H Mohapatara 2000 *Current Science* 78 967-973.
[22] Sari L A, W H Satyantini, A Manan, K T Pursetyo, N N Goddess 2018 *IOP Conference Series: Earth and Environmental Science* 137 012-029.
[23] Masithah E D, L A Sari, W H Satyantini, A T Mukti 2012 *Scientific Journal of Fisheries and Marine* 1 3.
[24] Sari L A, K T Pursetyo, S Arsad, E D Masithah, E Setiawan, M Affandi 2019 *The Effect Of Nutrient Abundance On Distribution Of Cyanobacteria And Chlorophyll-A In Sedati Water, Sidoarjo* (Poll Res) 38 S27-S32.
[25] Sari L A, P D W Sari, D D Nindarwi, S Arsad, M Affandi 2019 *Eco. Env. & Cons.* 25 S26-S31.
[26] Rice E W, R B Baird, A D Eaton, L S Clesceri 2012 *Standard Methods for the Examination of Water and Wastewater, 22th Edition* (Washington DC: American Public Health Association APHA) p 1496.
[27] Kusrininingrum 2008 *Basic Design of Experiments and completely randomized design. Faculty of Veterinary Medicine* (Surabaya: Airlangga University) p 53-92.
[28] Igwegbe A O, C H Agukwe, C A Negbenbor 2013 *Journal of Engineering and Science* 2 1-5.
[29] Ai N S 2012 *Scientific Journal of Science* 12 28-34.
[30] Sullivan J K 2000 *Marine and Freshwater Research* 28 739-743.