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Potential concentration of heavy metal copper (cu) and microalgae growth *Spirulina plantesis* in culture media

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Abstract. Water is an important environmental component for life. Heavy metal water pollution comes from many industries. Heavy Metals Copper (Cu) is one of several other heavy metals that are harmful to living things. One way to anticipate the increased pollution of heavy metals Copper (Cu) in waters is bioremediation using microalgae. This study aims to determine the ability of Spirulina plantesis in absorbing heavy metals Copper (Cu) and to determine the influence of heavy metal Copper (Cu) on the growth of Spirulina platensis. This study used an experimental method with Completely Randomized Design (RAL) consisting of four treatments and five replicates, namely A (S. platensis 0 ppm), B (S. platensis 1 ppm), C (S. platensis 3 ppm), D (S. Platensis 5 ppm). The results showed that Spirulina platensis was able to absorb heavy metal of Copper (Cu) so that it can be used as a heavy metal bioremediation agent. On treatment B (1 ppm) absorption of 87,719%, C (3 ppm) equal to 97,886% and D (5ppm) equal to 95,872 % Growth with the addition of Cu affects Spirulina platensis growth

1. Introduction

Water is essential for life environment. Living creatures on this earth can not be separated from the need of water. The quality and quantity of water can be a threat if they are not available in the correct condition [1]. If this condition is left unchecked, the future will worsen the condition of the environment and harm to human health due to increased pollution status and accumulation of heavy metals in biota [2].

Cu is one kind of some other heavy metals that are harmful to living things. A heavy metal pollutant for plants, animals and human health. In humans, copper poisoning can lead to hepatic cirrhosis, brain damage and a decline in kidney function [3]. Based on Threshold Limit Values (TLV) areRegulation of the Government of the Republic of Indonesia No. 82 of 2001 on water quality management and water pollution control with Cd metal content limit should not exceed 0.02 mg/L in a body of water.

Bioremediation is one of the alternatives for dealing with heavy metal toxicity. Bioremediation is the use of microorganisms that have been selected to be grown on a specific pollutants in an effort to reduce levels of pollutants [4]. Microalgae is the lowest level plants that have a very important role in

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aquatic ecosystems as primary producers and suppliers of oxygen waters. Microalgae are a good bioremediator [5].

Spirulina platensis is one type of algae with a single cell which belongs to a class Cyanophyceae. These algae has a high ability to bind metal ions from solution and adsorb heavy metals because the algae are functional groups that can perform the binding with metal ions. The functional groups, especially carboxyl groups, hydroxyl, amine, sulfudril imadazol, sulfate and sulfonate contained in the cytoplasm of the cell wall. *Spirulina platensis* has the ability to absorb heavy metals. For that to know the ability of *Spirulina platensis* in different concentrations decline and the impact of concentration on growth. Therefore, this study was conducted to determine a decrease in the concentration of heavy metals (Cu) to the culture medium and to determine the effect of the concentration of copper (Cu) on the growth of Spirulina platensis.

2. Materials and methods

2.1. Place and time of research

This study was conducted from April to May 2017 in the Faculty of Fisheries and Marine Laboratory, University of Airlangga. Examination of the content of copper (Cu) in water culture medium Spirulina platensis conducted in Research and Industry Standards (Baristand) Surabaya.

2.2. Tools and materials

The equipment used in this research is autoclave, filter bag, digital scale, measuring flask, measuring cup, syringe, 1L treatment glass bottle, volume pipette, 40 watt fluorescent lamp, microscope, drop pipette, sedgewick rafter, hand tally counter, thermometer, pH meters, DO meters, refractometers, and aeration equipment. The tool used to check the content of Copper (Cu) is the Simatsu AA 6200 Flame Atomic Absorption Spectrometry (ASS) with a Limit Of Quantification (LOQ) <0.0078.

The research materials used were sea water, chlorine, Na-Thiosulfate, liquid soap, aluminum foil, heavy metal Copper (Cu), aquades, pure culture Spirulina platensis obtained from the Central Office of Brackish Aquaculture Fisheries (BBPBAP) Jepara, and Walne media .

2.3. Work procedures

This research was conducted experimentally, the research design used was a Completely Randomized Design (CRD) consisting of four treatments with five replications, namely:

- A: S. platensis without the addition of Cu
- B: S. platensis with the addition of Cu 1 ppm
- C: S. platensis with the addition of Cu 3 ppm
- D: S. platensis with the addition of 5 ppm Cu

Provision of heavy metals Copper (Cu), amounting to 1.3, and 5 ppm based on the results of research Prambodo et al. [6] that *Spirulina platensis* was able to reduce the concentration of Copper (Cu) and grow in culture media that were given 1.3, and 5 ppm heavy metals.

2.4. Tools and media of sterilization of culture

Equipment is used before sterilization, all tools are first washed with liquid soap and rinsed thoroughly. Then the equipment was put into a 1 atm pressure autoclave, at 121°C for 15 minutes [7]. For 1 L treatment bottles and analytical sample bottles washed thoroughly with liquid soap then soaked with chlorine, rinsed and dried.

Further sterilization of sea water will be used as a culture medium *Spirulina platensis*, The sea water is filtered by the filter bag and then sterilized using 60 ppm chlorine solution for 24 hours and neutralized with a solution of 20 ppm Na-thiosulfate until no smell of chlorine [8].

2.5. Preparation of solutions stock Cd

Manufacture of heavy metal stock solutions Cu. 1 ppm Copper (Cu) solution made is a stock solution with a concentration of 10 ppm. Stock solution is made by weighing 5 mg of $CuSO_4$ then dissolving it in 500 ml of distilled water in a measuring flask. The volume of solution taken to obtain a concentration of 1 ppm in Copper (Cu) stock solution is 50 ml. For a concentration of 3 ppm weighing 1.66 mg of CuS04 then dissolving in 500 ml distilled water in a volumetric flask. The volume taken to obtain 3 ppm in Copper (Cu) stock solution is 16.6 ml. At 5 ppm Cu stock solution makes 1000 ppm stock solution. Generate a calculation of 5 mg which is then dissolved in 500 ml of distilled water. The volume of the ocean taken to obtain Copper (Cu) concentration of 5 ppm is 0.5 ml.

Taking copper stock (Cu) which will be treated using the following formula [9].

$$V1 \times N1 = V2 \times N2$$

Information :
V1: volume of stock sought (mL)
N1: sought stock concentration (mg/L)
V2: known stock volume (mL)
N2: known stock concentration (mg/L)

2.6. Phytoplankton nannochloropsis oculata and chlorella vulgaris cultures

Culture media used in this study were 500 ml sterilized sea water and aerated. *Spirulina platensis* seedlings were inserted in bottles of each treatment with a density of 1x105 cells / ml based on Nisak [10]. Furthermore, in each culture medium each treatment was added with as much as 0.5 mL/L walne fertilizer. Culture is carried out for 7 days.

2.7. Measurement of copper (Cu) on culture media Spirulina platensis

Measurement Cudone before the start of culture on day 0 and after maintenance of *Spirulina platensis* culture on day 7. The content of Cu in sea water in a test media by Flame Atomic Absorption Spectrometry (ASS) Simatsu AA 6200 with Limit Of Quantification (LOQ) <0.0078 in Research and Industry Standards (Baristand) Surabaya.

2.8. *Percentage calculation of absorption and density of phytoplankton*

Data retrieval absorption capability of Copper (Cu) is performed on day 0 and day 7. According Wiyarsi and Priyambodo [11], calculation of absorption efficiency using the following formula:

$$Eff = \frac{t0 - t7}{t0} \times 100\%$$

Information :
Eff: Absorption efficiency (mg /L)
t0 : Metal concentrations at baseline (mg / L)
t7 : Metal concentrations at the end of the study (mg / L)

Calculation of density performed daily for seven days to determine the growth of phytoplankton by using Sedgewick rafter.

$$\frac{\mathbf{nA + nB + nC + nD + NE}}{\mathbf{5 \times 4 \times 105}}$$

$$N = n\left(\frac{s}{lp}\right) x \left(\frac{p}{v}\right)$$

Information :
N = Density of Spirulina platensis (units / ml)
N = Number of plankton in the 10 field of view (Units)
S = Number of field perspective on Sedgewick Rafter
Ip = Amount used visual field
Subsample P = Volume (ml)
V = sample volume (ml)

2.9. Data analysis
Analysis of data using Analysis Of Varian (ANOVA) to determine the differences between treatments. If the treatment is given provide a significantly different effect will be continued with Duncan Multiple Range Test (*Duncan's Multiple Range Test*) To determine differences among treatments one with the other treatments [12].

3. Results and discussion

3.1. Results

3.1.1. Ability nannochloropsis oculata and chlorella vulgaris in absorbing heavy metal Cadmium
Results of analysis of the heavy metal content of copper (Cu) beginning and end of the culture medium Spirulina platensis for seven days showed a decrease in the concentration of heavy metals copper (Cu), The content and the percentage of water absorption of Cu in maintenance media can be seen in Table 1.

Table 1. The content and the percentage of water absorption of Cu in maintenance media.

Treatments	Percentage ± SD
A	$0^a \pm 0$
B	87.72 ± 2.01
C	97.88 ± 0.60
D	95.87 ± 0.54

Description: Description: A (S. platensis 0 ppm), B (S. platensis 1 ppm), C (S. platensis 3 ppm), and D (S. platensis 5 ppm). Different superscripts in the same column indicate significant differences ($p < 0.05$).

Statistical test results analysis of variants (ANOVA) interval of 95% on Spirulina platensis to heavy metals copper (Cu) showed results significantly different ($P < 0.05$). The highest percentage amount obtained in the treatment of heavy metals the addition of copper (Cu)) 3 ppm with an average percentage of 97.886%. Meanwhile, the lowest in treatment without the addition of the heavy metals copper (Cu) is 0%. While for the treatment of 1 ppm and 5 ppm respectively has a percentage reduction of heavy metals copper (Cu) 87.719% and 95.872%.

3.1.2. Growth of Spirulina platensis
Spirulina platensis growth is calculated every day until the seventh day. In this growth, we also calculated density of *Spirulina platensis*. All treatments are calculated density is well treated by the addition of heavy metal and without the addition of heavy metals, growth data retrieval aims to determine the absorption of heavy metals and heavy metal influence on *Spirulina plantesis*. Average density data of *Spirulina platensis* are presented in Table 2.

Table 2. The average growth of Spirulina platensis for 7 days.

Treatments	Density of *S. plantesis* (10^3 cell/m) in day to-						
	1	2	3	4	5	6	7
A	1	1.216	1.342	3.362	3.904	4.902	5.044
B	1	1.196	1.294	3.422	3.516	4.734	4.904
C	1	1.008	0.984	1.454	3.198	3.556	4.220
D	1	1.026	1.248	1.796	3.070	2.658	3.146

Note: A (*S. plantesis* 0 ppm), B (*S. plantesis* 1 ppm), C (*S. plantesis* 3 ppm), and D (*S. plantesis* 5 ppm)

3.1.3. Water quality
The results of the measurement range of the average water quality culture media *Spirulina platensis* during the study are presented Table 3.

Table 3. The average value of water quality culture media *Spirulina platensis*

Parameter	Range
Temperature	29-33
DO (mg/L)	5-6
pH	7
Salinity (ppt)	30

3.2. Discussion

The results of water measurements in Spirulina plantesis culture media containing heavy metals Copper (Cu) of 1, 3, and 5 ppm have decreased the concentration of heavy metals. In 1 ppm heavy metals, the reduction of heavy metals to 0.13 ppm, for 3 ppm the reduction of heavy metals to 0.05, while in the 5ppm treatment the reduction of heavy metals to 0.188 Based on the analysis of initial and final heavy metal content of Copper (Cu) on Spirulina plantesis culture media during the study showed a decrease in the content of heavy metals Copper (Cu). The decrease occurred because the heavy metal Copper (Cu) undergoes a binding process with a functional group by *Spirulina plantesis*. Based on the table above shows that the best percentage is the absorption of heavy metals with a concentration of 3 ppm. The difference in absorption was 2,650 with a percentage of 97,886%.

The process of reducing the concentration of Cu in water that occurs in the treatment of Cu 1 ppm, 3 ppm and 5 ppm shows that there is absorption of Cu in the water by *Spirulina plantesis* cells. Percentage reduction in Cu content of 87.719%, 97.886%, and 95.872%, this is supported by the statement of Soeprobowati and Hariyati [5] states that *Spirulina plantesis* which is cultured into heavy metals can reduce concentrations up to 88% in Pb, 85% in Pb, 85% in Cd, 89% in Cu, and 88% in Cr.

The growth of *Spirulina plantesis* was observed for 7 days by the growth of *Spirulina plantesis* experienced a final phase (stationary phase) within a period of 6 days. This study was conducted 7 days because it was to determine cell changes after *Spirulina platensis* experienced the final phase (stationary phase) and accumulated heavy metal Cu in the cells.

The relationship of the maximum growth rate of *Spirulina plantesis* with the treatment of different heavy metal concentrations of Cu shows that the treatment with the addition of 1 ppm heavy metal is needed for growth but the higher the concentration of heavy metal Cu given, the lower the density of *Spirulina plantesis*. Torres [13] said heavy metals Cu is an essential metal and micronutrients needed for growth and development.

At 5 ppm addition of heavy metal Cu growth was inhibited but Spirulina plantesis did not experience death. This occurs due to heavy metal Cu concentrates where heavy metals inhibit respiration for photosynthetic microalgae. In addition, the sensitivity of microalgae to different heavy metals of each species interferes with physiological processes and inhibits photosynthesis. Pollutants entering mesophyll cells will affect the molecular level causing changes in stomatal response, chloroplast structure, CO_2 fixation [14].

The peak of growth in each treatment also experienced differences. Spirulina plantesis without the addition of Cu (A) reached the peak of population on the 7th day with a density of 5.0 × 105. *Spirulina plantesis* with the addition of Cu 1 ppm (B) reached the peak of the population that is on the 7th day with a density of 4.9 × 105 and the addition of 3 ppm 4.22 × 105 compared with the addition of Cu 5 ppm which is less that is 3.1 × 105.

The results of water quality data during the study showed that the condition of water quality parameters of culture media such as temperature, DO, pH, salinity are still in optimal condition. The temperature during Spirulina platensis culture that lasted 7 days ranged from 29 to 33°C. DO is dissolved oxygen found in waters where dissolved oxygen is used for respiration. During the study dissolved oxygen (DO) was obtained in the range of 4-6 mg / l. This condition is still optium for the growth of Spirulina platensis. The degree of acidity (pH) when culture is at 7-8. Culture salinity at 30 ppt.

4. Conclusion

Spirulina plantesis can absorb Heavy Metal Copper (Cu) in all treatments. The best absorption of treatment C is by giving 3 ppm heavy metals with a percentage reaching 97.886%. Heavy metal Copper (Cu) affects the growth of Spirulina plantesis in the treatment of 5 ppm Copper (Cu) because this concentration is toxic to the growth of *Spirulina plantesis*. The results of the study are expected to be used to reduce waste containing heavy metal Copper (Cu) in the waste management installation before being discharged free of charge so that the waters remain sustainable and can be utilized sustainably. And research needs to be done with other heavy metals or combine heavy metals to be absorbed.

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