HAYATI
Journal of Biosciences

Vol. 32 No. 2, March 2025 387-394
DOI:10.4308/hjb.32.2.387-394
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

Check for updates

OPEN ACCESS

Effects of Copper on *Euglena* sp. Local Strains and Remediation Capabilities

Witia Ardipeni[1], Eko Agus Suyono[2*], Nugroho Dewayanto[3], Na'imatul Mufida[4], Tia Erfianti[2], Ria Amelia[2], Dedi Kurnianto[2]

[1]*Department of Environmental Science, Graduate School, Universitas Gadjah Mada, Sleman 55281, Indonesia*
[2]*Department of Biology, Faculty of Biology, Universitas Gadjah Mada, Sleman 55281, Indonesia*
[3]*Department of Systems Engineering, Faculty of Engineering, Sleman 55281, Indonesia*
[4]*Department of Biotechnology, Graduate School, Universitas Gadjah Mada, Sleman 55281, Indonesia*

ARTICLE INFO

Article history:
Received July 24, 2024
Received in revised form September 12, 2024
Accepted November 6, 2024

KEYWORDS:
Euglena sp.,
Copper,
Growth,
Photosynthetic Pigment,
Superoxide Dismutase,
Bioremediation

ABSTRACT

One of the well-known industries in Yogyakarta is the Kotagede silver craft, which produces heavy metal waste, one of which is copper. The copper content in the liquid waste of electroplating Kotagede silver crafts is 4.628 mg/L. At the same time, Indonesian Government Regulation No. 22 (2021) stipulates a critical limit of Copper (Cu) content in river and lake water, which is 0.2 mg/L. The purpose of the study was to analyze the effects of copper on local strains of *Euglena* sp. (growth, photosynthetic pigment production, and superoxide dismutase enzyme) as well as the effectiveness of *Euglena* sp. in copper remediation. The study was conducted by adding a copper solution obtained from $CuSO_4 \cdot 5H_2O$ to the culture medium of *Euglena* sp. The parameters observed in the study were growth (number of cells and biomass), photosynthetic pigment content (chlorophyll a, chlorophyll b, carotenoid total), Superoxide dismutase enzyme of *Euglena* sp. as well as the effectiveness of *Euglena* sp. in copper remediation. The results showed that copper inhibited the growth of *Euglena* sp. The highest growth was the control treatment (without adding copper to the culture medium). Likewise, the content of photosynthetic pigments and the culture of *Euglena* sp. exposed to copper were lower when compared to the control culture. The activity of the SOD enzyme is increased in cultures exposed to copper. The result of the research is Cu metal absorption efficiency by *Euglena* sp. by 21.93%, 10.93%, and 9.47% for a Cu concentration of 10 ppm, 15 ppm, and 20 ppm.

1. Introduction

One of the water problems in Indonesia is heavy metal pollution. Heavy metals are nonbiodegradable and have a long-lasting persistence that can harm human health and ecosystems (Shah 2023). In Yogyakarta, Indonesia, there is a silver craft industry that produces liquid waste. Research conducted by Sumiyati *et al.* (2009) showed that the concentration of Cu metal in the liquid waste of electroplating Kotagede silver crafts was tested at 4.628 mg/L. Indonesian Government Regulation No. 22 (2021) attachment 6 concerning the Implementation of Environmental Protection and Management stipulates a critical limit of Copper (Cu) content in river and lake water, which is 0.2 mg/L.

Copper (Cu) is one of the essential heavy metals for living things to a certain extent. Copper functions as a catalytic and structural cofactor for enzymes that function in energy generation, iron acquisition, oxygen transport, cell metabolism, peptide hormone maturation, blood clotting, and signal transduction (Kim *et al.* 2008). Continuous copper release is an essential problem in the case of phytotoxicity, namely excessive production of ROS and damage to carbohydrates, lipids, proteins, and DNA (Rehman

* Corresponding Author
E-mail Address: eko_suyono@ugm.ac.id

et al. 2019). In humans, copper that exceeds the threshold value of copper levels that can be consumed can cause vomiting, diarrhea, and wilson disease (Roychoudhury *et al.* 2016).

Phycoremediation is the use of macroalgae or microalgae for environmental remediation (Olguin 2003). Microalgae are organisms that can grow in the aquatic environment and use light and carbon dioxide (CO_2) to make biomass and act as oxygen producers in the waters (Tan *et al.* 2011). Microalgae can be used as a remediation agent for pollutants found in wastewater. One type of microalgae that can be used for bioremediation is *Euglena*. Winters *et al.* (2017) conducted a study using *Euglena gracilis* to remove copper and nickel. The results of their research showed that was able to remove 58±3% of copper with initial concentrations of 20 µg/L and 50 µg/L. Microalgae can adapt to extreme environmental conditions, such as in a very acidic environment. Euglenaceae family is one of the microalgae that can live in extreme environments and convert sunlight into chemical energy efficiently. Dieng wetland has an acidic pH, of ± 3.5 (Erfianti *et al.* 2023).

The high level of copper in the wastewater of the Kotagede silver craft industry will be dangerous if it is discharged into the waters without prior management. It will have a damaging impact on organisms in the waters and humans and disrupt the ecosystem. *Euglena* is widely found in freshwater waters, so it is expected to be able to absorb copper metal. This study aims to figure out the effects of copper metal on *Euglena* sp. and its ability to bioremediate copper in waters.

2. Materials and Methods

2.1. Culture of *Euglena* sp.

Euglena sp. local strain is a microalga of the genus *Euglena* that was isolated in the Dieng Peatland area, Wonosobo, Central Java, with a pH of ± 3.5 (Erfianti *et al.* 2023). The culture medium and nutrients used were Cramer-Myers medium. *Euglena* sp. was cultured in a 1.5 L bottle. The ratio of *Euglena* sp. to the culture medium was 2:3 with an optical density of 680 nm of 0.326. The pH of the first cultivation was ±3.5. Each treatment was conducted three times. The treatment conducted was control (without copper) and copper solution (10 ppm, 15 ppm, and 20 ppm). *Euglena* sp. culture was performed for nine days.

2.2. Copper Metal Solution

The copper metal solutions used in this study were 10 ppm, 15 ppm, and 20 ppm. The metal solution was melted by dissolving 3.9 g of $CuSO_4 \cdot 5H_2O$ in 1 L of aquadest, resulting in a copper solution of 1,000 ppm as a stock solution. Copper metal solutions of 10 ppm, 15 ppm, and 20 ppm were made using the formula of $V_1 \times N_1 = V_2 \times N_2$ where V_1: the volume of stock sought (ml); V_2: the known volume (ml); N_1: the sought-after stock concentration (mg/L); N_2: known stock concentration (mg/L).

2.3. Growth of *Euglena* sp.

The counting of the number of microalgae was conducted daily using a Neubauer hemocytometer and a microscope. According to Hakim *et al.* (2023), the calculation of the number of cells was found in Equation 1. The calculation of copper inhibition on growth, according to Purbonegoro *et al.* (2018), was found in Equation 2.

$$\text{the number of cell/ml} = \frac{\text{the number of cells counted}}{\text{counted squares}} \times 10{,}000 \quad (1)$$

Information:
Counted square: 4; Number of cells counted: Cells found at observation

$$\text{Inhibition of Cu (\%)} = \frac{C - T}{C} \times 100\% \quad (2)$$

Information:
C: Control treatment; T: Cu Treatment

Biomass calculations were conducted every two days. Whatman filter paper GF/C was dried in a 100°C oven for 1 hour. A culture sample of *Euglena* sp. 10 ml was taken and then filtered with Whatman filter paper GF/C. After that, it was dried in the oven for 1 hour at a temperature of 100°C. The biomass was weighed using a digital scale. The biomass calculation was found in Equation 3.

$$Bi = W_1 - W_0 \quad (3)$$

Information:
Bi : Biomass (mg/L); W_0: Filter paper weight; W_1: Weight of microalgae sediment + oven baked filter paper

2.4. Photosynthetic Pigments

The calculation of photosynthetic pigments was conducted every two days. The calculation of *Euglena* sp. photosynthetic pigment content was performed based on modifications from Maghfiroh *et al.* (2023). Culture samples of 2 ml were centrifuged at a rate of 4,000 g for 5 minutes. The supernatant was discarded, and the pellets were taken. The pellets were extracted with Methanol and then left for 24 hours in the dark condition using aluminium foil in the refrigerator. The extract was placed on a cuvette of 2 ml. The cuvette was inserted into a Uv-vis spectrophotometer, and its absorption was measured at wavelengths of 652 nm, 665 nm, and 480 nm. The results of spectrophotometry were included in Equation 4 for chlorophyll a, Equation 5 for chlorophyll b, and Equation 6 for Total carotenoid content.

$$\text{Chlorophyll a} = -8.0962 \times A_{652} + 16.5169 \text{ x } A_{665} \quad (4)$$

$$\text{Chlorophyll b} = 27.4405 \times A_{652} - 12.1688 \times A_{665} \quad (5)$$

$$\text{Total carotenoid content} = 4 \times A_{480} \quad (6)$$

2.5. Superoxide Dismutase

The calculation of the Superoxide Dismutase (SOD) enzyme was done once on the last day. The culture sample of *Euglena* sp. 15 ml was centrifuged at a rate of 3,500 g for 15 minutes. In the pellets, 1 ml of sodium phosphate buffer was added and centrifuged for 15 minutes at a speed of 10,000 rpm, the temperature was set at 4°C. The results in the form of supernatants were stored to measure SOD activity in *Euglena* sp. by the modified pyrogallol method from Marklund and Marklund (1974). The principle of dismutation of reactive oxygen species by SOD metalloenzyme was had to the molecular nature of free oxygen, which has two unpaired electrons and parallel rotation. Free oxygen molecules prefer univalent reduction because of the limited rotation when reduced with electron pairs. SOD was able to quickly dismute univalent reduction of Oxygen O_2^- (Marklund and Marklund 1974). The composition solution in Table 1 was inserted into the cuvette. The Uv-vis spectrophotometer was set to kinetic mode, and the absorbance was set at 325 nm, with a measurement time duration of 5 minutes and 1-minute intervals. The calculation of the superoxide dismutase enzyme was found in Equation 7.

$$\text{SOD} = \left(\frac{\frac{dAb - dAs}{dAb} \times 100\%}{50\%} \right) \times 1.018 \times \frac{1}{8} \quad (7)$$

Information:
dAb: Delta of the blank solution (average result of the absorption measurement of the blank solution); dAs: Delta of the sample solution (average of the results of the sample solution absorbance measurement)

2.6. Effectiveness of Copper Remediation

The calculation of copper remediation was conducted once on the 9th day. Testing of copper metal uses Atomic Absorption Spectroscopy (AAS) performed by a third party. According to Afandi *et al.* (2014), the calculation of the remediation efficiency of heavy metals uses Equation 8.

$$\text{Eff} = \frac{C_0 - C_1}{C_0} \times 100\% \quad (8)$$

Information:
Eff: Absorption efficiency; C0: Concentration of precious metals; C1: Concentration of metals after absorption

2.7. Data Analysis

The differences between the treatments were analyzed using the ANOVA (Analysis of Variance) test with significant (α) value of 0.05 and a further Tukey test was conducted. The Kruskal Wallis non-parametric test analyzed data with abnormal distribution.

3. Results

3.1. Growth of *Euglena* sp.

The study was conducted by cultivating *Euglena* sp. for nine days with Cu metal treatment (10 ppm, 15 ppm, and 20 ppm) and without metal treatment (control). The growth of *Euglena* sp. is known by calculating the number of cells and biomass. The growth of *Euglena* sp. based on the number of cells can be seen in Figure 1, while based on biomass is found in Figure 2. In addition

Table 1. Composition of solution in measurement of SOD enzyme

Solution type	Composition	Quantity (ml)
Blank spectro	Buffer Tris-EDTA	1
	Aquades	0.008
Ablank	Buffer Tris-EDTA	1
	Aquades	0.008
	Pyrogallol	0.010
Sample	Buffer Tris-EDTA	1
	Aquades	0.008
	Pyrogallol	0.010

to inhibiting growth, copper metal causes *Euglena* sp. cell size to be larger compared to the control treatment. A comparative picture of the morphology of *Euglena* sp. is shown in Figure 3.

The growth of microalgae is also affected by several environmental factors, such as light intensity, salinity, pH, and temperature. The measurable environment parameters during the study are shown in Table 2.

3.2. Photosynthetic Pigments

The effects of copper on the observed photosynthetic pigments are chlorophyll a, chlorophyll b, and total carotenoid content. The exposure of copper results in decreased production of photosynthetic pigments. The difference in photosynthetic pigment content in control cultures and cultures exposed to copper metal is shown in Table 3.

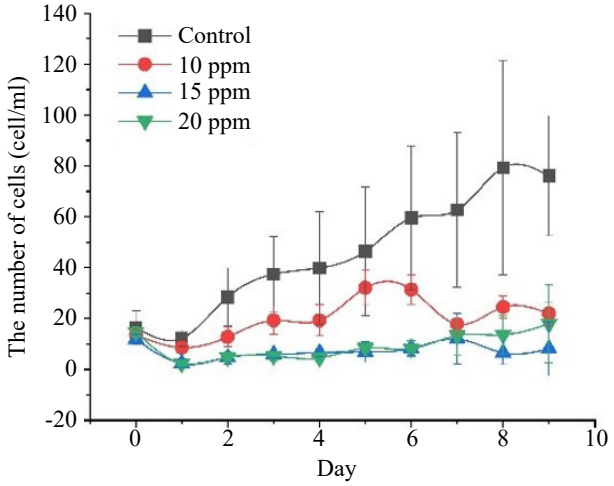

Figure 1. Growth of *Euglena* sp. based on cell count. Copper inhibition against *Euglena* sp. growth at 10 ppm treatment was 31%, 15 ppm was 81%, and 20 ppm was 76%. The error bar indicates the standard deviation

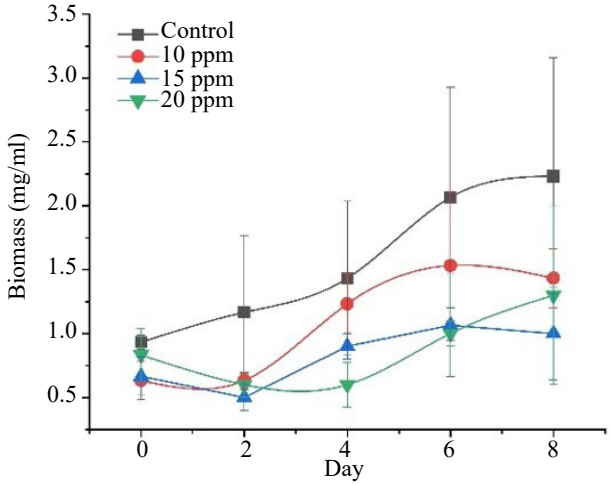

Figure 2. Growth of *Euglena* sp. according to biomass content

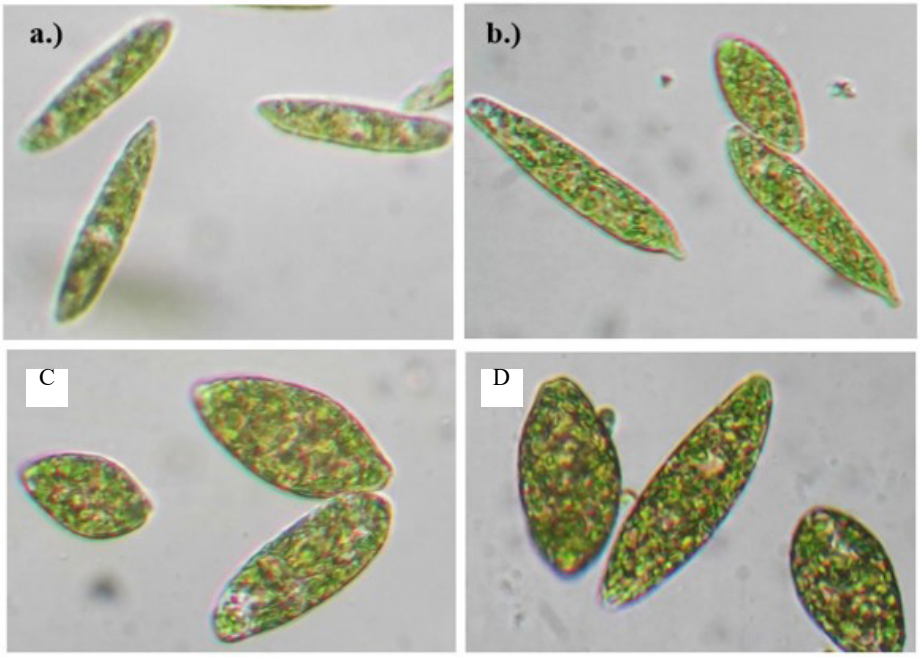

Figure 3. Morphological observation of *Euglena* sp. under an inverted microscope (40x) (A) control, (B) Cu metal 10 ppm, (C) Cu metal 15 ppm, (D) Cu metal 20 ppm. Source: Personal documents

Table 2. Average environmental parameters during the cultivation of *Euglena* sp.

Environmental parameters	Average Cu metal treatment			
	Control	10 ppm	15 ppm	20 ppm
pH	3.4	3.4	3.5	3.5
Temperature (°C)	29.4	28.6	29.9	29.9
Light intensity (Lux)	6969	7987	7718	7718
Salinity	0.45	0.43	0.41	0.41

Table 3. Photosynthetic pigment content value on day 8

Environmental parameters	Treatment			
	Control	10 ppm	15 ppm	20 ppm
Chlorophyll a	3.58±1.66[a]	2.16±0.6[a,b]	0.71±0.54[b]	1.08±0.65[b]
Chlorophyll b	0.34±0.19	0.27±0.09	0.09±0.06	0.23±0.13
Carotene	0.87±0.33[a]	0.52±0.11[a,b]	0.17±0.12[b]	0.25±0.14[b]

3.3. Superoxide Dismutase Enzyme

Copper application to *Euglena* sp. culture media affects the number of SOD enzymes. Exposure to copper leads to an increase in ROS production. SOD is an antioxidant that can inhibit oxidation due to ROS to reduce cell damage due to oxidative stress. Therefore, the SOD enzyme is increased due to copper exposure. The antioxidant activity of *Euglena* sp., SOD, against Cu exposure is seen in Figure 4.

3.4. Copper Metal Remediation

The effectiveness of copper metal remediation by *Euglena* sp. is known through the concentration of copper left in the culture medium on the last day of cultivation (day 9). The effectiveness of copper metal remediation is shown in Figure 5.

4. Discussion

4.1. Growth of *Euglena* sp.

Copper metal inhibits the growth of *Euglena* sp. The results showed that the control culture had the highest growth compared to the culture exposed to copper (Figure 1 and 2). Microalgae growth based on cell number and biomass showed the same thing. Copper inhibited the growth of *Euglena* sp. and copper concentration also had an effect. Copper inhibition against *Euglena* sp. growth at 10 ppm treatment was 31%, 15 ppm was 81%, and 20 ppm was 76%. This also happened in the research of Hamed *et al.* (2017), which showed that microalgae *Chlorella sorokiniana* and *Scenedesmus acuminatus* exposed to

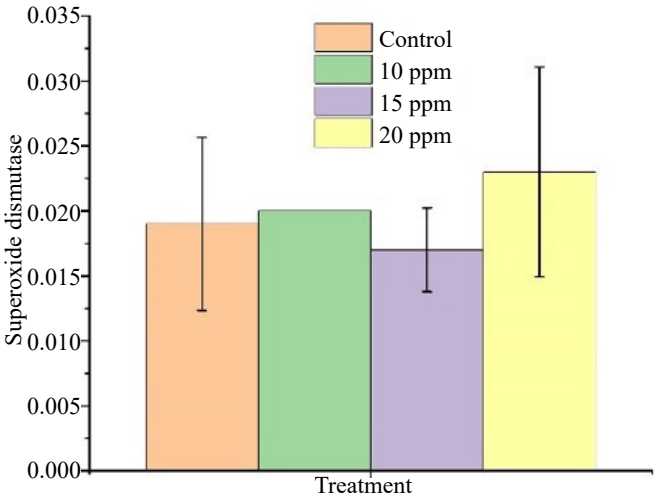

Figure 4. Effect of Cu on SOD enzyme *Euglena* sp. SOD enzyme testing was conducted on day 9. The SOD enzyme value in each treatment was 0.019±0.007 (control); 0.02±0.000 (10 ppm); 0.017±0.003 (15 ppm); and 0.023±0.008 (20 ppm). SOD testing was conducted on day 9. The Kruskal Wallis test of the SOD enzyme showed that there was no significant difference in each treatment (sig value>0.05). Error bars show standard deviations

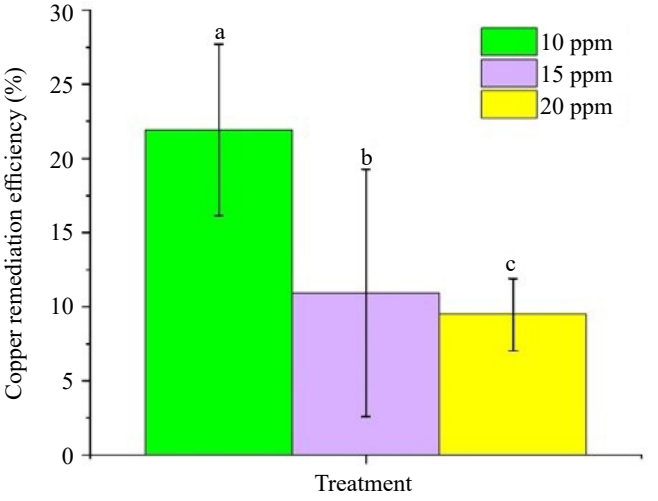

Figure 5. Copper remediation efficiency by *Euglena* sp. Copper measurements in the media were carried out on the 9th day. The ratio of media to culture was 3:2 in 1.5 L. The pH of the culture on day 9 was 3.3 (10 ppm) and 3.4 (15 ppm and 20 ppm). The pH at the beginning of the culture is made the same, which is ±3.5. The Cu metal removal efficiency was 21.93% (10 ppm), 10.93% (15 ppm), and 9.47% (20 ppm). Error bars show standard deviations. Based on the ANOVA test and Tukey test, the 10 ppm, 15 ppm, and 20 ppm treatment was significantly different

copper metal grew under culture in the control medium. The results of their study showed that the control culture had higher growth compared to the culture exposed to copper with concentrations of 25 µM, 50 µM, 100 µM, and 400 µM.

Microalgae respond to copper-rich environmental conditions by producing O_2^- mediated ROS through redox reactions, mainly by the production of singlet oxygen ($^1O_2$), superoxide anion radicals (O_2), hydroxyl radicals (OH), perhydroxy radicals (HO_2), and hydrogen peroxide (H_2O_2). ROS is a prooxidant that causes oxidative stress in organisms. Increased ROS levels cause damage to cells, such as (1) DNA: strand breaking, depurification and deirimidation, base mutations, protein DNA cross-linking); (2) Protein: amino acid modification, peptide breakage, improve proteolytic degradation, enzyme inactivation; (3) lipids: improve membrane fluidity and permeability, lipid chain breaking. (Gauthier *et al.* 2020; Danouche *et al.* 2022) The impact of high Cu absorbed by microalgae causes the accumulation of photosynthetic products in cells through carbon fixation (Cavalletti *et al.* 2022). Carbon fixation produced in photosynthesis cannot be released or used in cell division, so photosynthetic products accumulate and cause cells to enlarge (Purbonegoro *et al.* 2018).

The growth of cultures exposed to Cu is 20 ppm higher than cultures of 15 ppm, which is likely to cause the hormesis phenomenon. Hormesis is a process in which exposure to chemicals or environmental factors in low doses can be damaging. In contrast, at higher doses, it induces beneficial effects that are adaptive in cells or organisms (Mattson 2008). At a concentration of 20 ppm, copper may trigger an optimal stress response, so the microalgae activate biochemical pathways that allow them to grow better than at lower concentrations (15 ppm), where the mechanism has not been fully activated. Copper exposure also causes the size of *Euglena* sp. cells to enlarge (Figure 3). Cells in the 15 and 20 ppm cultures were larger when compared to the 10 ppm and control cultures. Copper metal at certain concentrations is needed by phytoplankton for the process of photosynthesis and respiration (Cavalletti *et al.* 2022). The high concentration of copper inhibits the growth of microalgae because it inhibits the process of cell division. The impact of the high amount of copper absorbed by microalgae causes the accumulation of photosynthetic products in the cell through carbon fixation (Cavalletti *et al.* 2022). Carbon fixation produced in photosynthesis cannot be removed or used in cell division. Therefore that, photosynthetic products accumulate and cause cells to enlarge (Purbonegoro *et al.* 2018). The results of a study from Purbonegoro *et al.* (2018) show the same, where the microalgae *Pavlova* sp. exposed to copper with a concentration of 98 µg/L for 96 hours is 7.26 µm, larger when compared to control cultures which are only 5.4 µm.

4.2. Photosynthetic Pigments

The effect of Cu exposure on the growth of *Euglena* sp. also affects the content of photosynthetic pigments. The difference in the content of photosynthetic pigments in the control culture and the culture exposed to Cu metal is shown in Table 3. Copper has oxidation numbers Cu (I) and Cu (II). The existence of these oxidation numbers makes copper play a role in the transport of photosynthetic electrons that are reduced and oxidized when electrons are transferred to the photosystem reaction centre I (PS I). On the other hand, Cu (II) can also combine with Mg^{2+}, a chlorophyll molecule, which has an impact on changes in the structure of chlorophyll, thereby causing disruption of the electron transport chain and decreasing the efficiency of photosynthesis. The excited energy from the chlorophyll change is likely transferred to oxygen, resulting in the production of singlet oxygen, one of the ROS which causes oxidative damage. In addition, high concentrations of Cu (II) inhibit photosynthesis, especially light reactions, thereby damaging photosynthetic organisms (Sabatini *et al.* 2009; Chen *et al.* 2016).

In the previous study, Hamed *et al.* (2017) showed that the chlorophyll (a+b) content in *S. acuminatus* and *C. sorkiniana* cultured by adding 50 µM and 25 µM copper for seven days was lower than the control of 46.3% and 41.7%. The decrease in carotene content affected by Cu exposure also occurred in the study of Hindarti and Larasati (2019) which showed that the control carotene content (1.86 mg/L) was greater than that of microalgae exposed to Cu with concentrations of 0.018 mg/L (1.53 mg/L); 0.032 mg/L (1.92 mg/L); 0.056 mg/L (1.4 mg/L); 0.1 mg/L (1.27 mg/L); and 0.18 mg/L (1.15 mg/L).

4.3. Superoxide Dismutase Enzyme

Exposure to Cu metal in *Euglena* sp. culture affects the amount of SOD enzyme. The antioxidant activity of *Euglena* sp. against Cu exposure is shown in Figure 4. The highest SOD enzyme is found in cultures exposed to 20 ppm of Cu. Copper's toxicity to microalgae is mainly related to free ions (Li *et al.* 2006). Superoxide dismutase catalyzes the neutralization of 2 superoxide radicals (O_2^-) with the addition of 2 hydrogen ions (H^+) to form hydrogen peroxide (H_2O_2) and oxygen (O_2) (Cirulis *et al.* 2013). Cu exposure results in increased H_2O_2 production and SOD activity. The results showed that the SOD enzyme functioned as a protector against

copper-induced oxidative stress. Hamed *et al.* (2017) and Li *et al.* (2006) in their study also showed the same result, namely that the SOD value in the treatment exposed to Cu was higher than the control. At 15 ppm treatment, SOD activity was lower than control because many cells died because they were unable to survive Cu stress. At exposure to high concentrations of Cu (II), the response in cells is not enough to prevent damage due to ROS, so cells become damaged and even die, causing enzyme production to decrease (Li *et al.* 2018).

ROS is not only formed due to exposure to metals. Activities in cells can also produce ROS. Microalgae are photosynthetic organisms and conduct aerobic metabolism. Aerobic metabolism will produce ROS, which is limited in number to specific cell organelles, such as chloroplasts, mitochondria, and peroxisomes. Under natural conditions, intracellular ROS concentrations in microalgae cells are constantly kept at a low equilibrium level by the ROS antioxidant system (Danouche *et al.* 2022).

4.3. Copper Metal Remediation

The effectiveness of Cu metal reduction is shown in Figure 5. Cu metal absorption efficiency by *Euglena* sp. is 21.93%, 10.93%, and 9.47% for a Cu concentration of 10 ppm, 15 ppm, and 20 ppm. This happens because the number of *Euglena* sp. cells at a concentration of 10 ppm is greater, and their growth is faster than that of cultures exposed to Cu concentrations of 15 ppm and 20 ppm. Biological absorption by algae occurs in two stages. First, there is physical adsorption at the cell surface, and then there is a slower bioaccumulation through internal transport and chelation (Zhou *et al.* 2012). As soon as the algae and metal come into contact, physical adsorption takes place quickly. Chemisorption is a term used to describe the slow process of bioaccumulation linked to metabolic activity.

Leong and Chang (2020), in their review, wrote that the adsorption of metals on the surface of microalgae is a rapid process and can occur through various pathways, namely the formation of covalent bonds between ionized cell walls and metals, the exchange of metal ions with cell wall cations and the binding of metal cations with negatively charged uronic acid from microalgae exopolysaccharides. In contrast, the process of accumulation of metals in cells occurs much more slowly. Metals are actively transported across the cell membrane and into the cytoplasm, followed by diffusion and further binding with the internal binding sites of proteins and peptides such as GSH, metal transporters, oxidative stress-reducing agents, and phytoketamine.

Research on the bioremediation of Cu heavy metals using microalgae has been conducted using different concentrations of metals and types of microalgae. The study of Budi *et al.* (2018) using *Spirulina platensis* to remediate Cu metal showed results at a concentration of 1 ppm absorption of 87.719%, a concentration of 3 ppm of 97.886%, and a concentration of 5 ppm of 95.872%. The results of Yusuf (2014) research show that *S. platensis* is able to reduce Cu concentration in culture media. Another study using the type microalgae type *Chaetoceros calcitrans* (Paulsen) Takano by Fitriyanto *et al.* (2016) showed that after seven days, the study was able to reduce Cu concentration by 20% (from 0.595 mg/L to 0.478 mg/L) within seven days with a total of 2.8×106 cells/ml.

The conclusion of this study is that exposure to copper metal causes growth inhibition, decreased production of photosynthetic pigments, and increased superoxide dismustase enzyme *Euglena* sp. Microalgae *Euglena* sp. are able to remediate copper heavy metals at concentrations of 10 ppm, 15 ppm, and 20 ppm with an efficiency level of 9-21%.

Acknowledgements

This manuscript is a part of first author's thesis. The authors thank to The Biotechnology Laboratory, Faculty of Biology, UGM University, for providing research facilities.

References

Afandi, A.Y., Soeprobowati, T.R., Hariyati, R., 2014. Pengaruh perbedaan kadar logam berat kromium (Cr) terhadap pertumbuhan populasi *Spirulina plantesis* (Gomont) geitler dalam skala laboratorium. *Jurnal Biologi*. 3, 1-6.

Budi, M.S.B., Rahardja, B.S., Mastihah, E.D., 2018. Potensi penurunan konsentrasi logam berat tembaga (Cu) dan pertumbuhan mikroalga *Spirulina plantesis* pada media kultur. *Jurnal Akuakultur Rawa Indonesia*. 6, 83-93. https://doi.org/10.36706/jari.v6i1.7152

Cavalletti, E., Romano, G., Palma, Esposito, F., Barra, L., Chiaiese, P., Balzano, S., Sardo, A., 2022. Copper effect on microalgae: toxicity and bioremediation strategies. *Toxics.* 10, 527. https://doi.org/10.3390/toxics10090527

Chen, Z., Song, S., Wen, Y., Zou, Y., Liu, H., 2016. Toxicity of Cu (II) to the green alga *Chlorella vulgaris*: a perspective of photosynthesis and oxidant stress. *Environmental Science and Pollution Research*. 23, 17910-17918. https://doi.org/10.1007/s11356-016-6997-2

Cirulis, J.T., Scott, J.A., Ross, G.M., 2013. Management of oxidative stress by microalgae. *Canadian Journal of Physiology and Pharmacology*. 91, 15-21. https://doi.org/10.1139/cjpp-2012-0249

Danouche, M., El Ghatchouli, N., Arroussi, H., 2022. Overview of the management of heavy metals toxicity by microalgae. *Journal of Applied Phycology*. 34, 475-488. https://doi.org/10.1007/s10811-021-02668-w

Erfianti, T., Maghfiroh, K.Q., Amelia, R., Kurnianto, D., Sadewo, B.R., Marno, S., Devi, I., Dewayanto, N., Budiman, A., Suyono, E.A., 2023. Nitrogen sources affect the growth of local strain *Euglena* sp. isolated from dieng peatland, Central Java, Indonesia, and their potential as bio-avtur. *IOP Conference Series. Earth and Environmental Science*. 1151, 012059. https://doi.org/10.1088/1755-1315/1151/1/012059

Fitriyanto, E.B., Soeprobowati, T.R., Hariyati, R., 2016. Kemampuan *Chaetoceros calcitrans* (Paulsen) takano dalam menurunkan kadar logam berat tembaga (Cu) pada limbah tekstil. *BIOMA*, 18, 102-106. https://doi.org/10.14710/bioma.18.2.102-106

Gauthier, M.R., Senhorinho, G.N.A., Scott, J.A., 2020. Microalgae under environmental stress as a source of antioxidants. *Algal Research*. 52, 102104. https://doi.org/10.1016/j.algal.2020.102104

Hakim, W.H.A., Erfianti, T., Dhiaurahman, A.N., Maghfiroh, K.Q., Amelia, R., Nurafifah, I., Kurnianto, D., Siswanti, D.U., Suyono E.A., Marno, S., Devi, I., 2023. The effect of IAA phytohormone (Indole-3-Acetic Acid) on the growth, lipid, protein, carbohydrate, and pigment content in *Euglena* sp. *Malaysian Journal of Fundamental and Applied Sciences*. 19, 513-524. https://doi.org/10.11113/mjfas.v19n4.2884

Hamed, S.M., Selim, S., Klöck, G., AbdElgawad, H., 2017. Sensitivity of two green microalgae to copper stress: growth, oxidative and antioxidants analyses. *Ecotoxicology and Environmental Safety*. 144, 19-25. https://doi.org/10.1016/j.ecoenv.2017.05.048

Hindarti, D., Larasati, A.W., 2019. Copper (Cu) and Cadmium (Cd) toxicity on growth, chlorophyll-a and carotenoid content of phytoplankton *Nitzschia* sp. *IOP Conference Series: Earth and Environmental Science*. 236, 012053. https://doi.org/10.1088/1755-1315/236/1/012053

Kim, B.E., Nevitt, T., Thiele, D.J., 2008. Mechanisms for copper acquisition, distribution and regulation. *Nature Chemical Biology*. 4, 176-185. https://doi.org/10.1038/nchembio.72

Leong, Y.K., Chang, J.S., 2020. Bioremediation of heavy metals using microalgae: recent advances and mechanisms. *Bioresource Technology*. 303, 122886. https://doi.org/10.1016/j.biortech.2020.122886

Li, M., Hu, C., Zhu, Q., Chen, L., Kong, Z. and Liu, Z., 2006. Copper and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in the microalga *Pavlova viridis* (Prymnesiophyceae). *Chemosphere*. 62, 565-572. https://doi.org/10.1016/j.chemosphere.2005.06.029

Li, X., Yang, W.L., He, H., Wu, S., Zhou, Q., Yang, C., Zeng, G., Luo, L., Lou, W., 2018. Responses of microalgae *Coelastrella* sp. to stress of cupric ions in treatment of anaerobically digested swine wastewater. *Bioresource Technology*. 251, 274-279. https://doi.org/10.1016/j.biortech.2017.12.058

Maghfiroh, K.Q., Erfianti, T., NurAfifah, I., Amelia, R., Kurnianto, D., Sadewo, B.R., Maggandari, R., Aji, B.R., Budiman, A., Suyono, E.A., 2023. The effect of photoperiodism on nutritional potency of *Euglena* sp. Indonesian strains. *Malaysian Journal of Nutrition*. 29. 453-466. https://doi.org/10.31246/mjn-2023-0004

Marklund, S., Marklund, G., 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Europe Journal of Biochemistry*. 47, 469-474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x

Mattson, M.P., 2008. Hormesis defined. *Ageing Research Reviews*. 7, 1-7. https://doi.org/10.1016/j.arr.2007.08.007

Olguín, E., 2003. Phycoremediation: key issues for cost-effective nutrient removal processes. *Biotechnology Advances*. 22, 81-91. https://doi.org/10.1016/S0734-9750(03)00130-7

Purbonegoro, T., Suratno, Puspitasari, R., Husna, N.A., 2018. Toxicity of copper on the growth of marine microalgae *Pavlova* sp. and its chlorophyll-a. *IOP Conference Series: Earth and Environmental Science*. 18, 012060. https://doi.org/10.1088/1755-1315/118/1/012060

Rehman, M., Liu, L., Wang, Q., Saleem, M. H., Bashir, S., Ullah, S., Peng, D., 2019. Copper environmental toxicology, recent advances, future outlook: a review. *Environmental Science and Pollution Research*. 26, 18003-18016. https://doi.org/10.1007/s11356-019-05073-6

Roychoudhury, S., Nath, S., Massanyi, P., Stawarz, R., Kacaniova, M., Kolesarova, A., 2016. Copper induced changes in reproductive functions: *in vivo* and *in vitro* effects. *Physiological Research.* 65, 11-22. https://doi.org/10.33549/physiolres.933063

Sabatini, S.E., Juarez, A.B., Eppis, M.R., Bianchi, L., Luquet, C.M., de Molina, M.D.C.R., 2009. Oxidative stress and antioxidant defenses in two green microalgae exposed to copper. *Ecotoxicology and Environmental Safety*. 72, 1200-1206. https://doi.org/10.1016/j.ecoenv.2009.01.003

Shah, M.P., 2023. *Phycoremediation Processes in Industrial Wastewater Treatment*. CRC Press, New York. https://doi.org/10.1201/9781003165101

Sumiyati, S., Dwi, S.H., Widya, H., 2009. Pemanfaatan Hydrilla (*Hydrilla verticillata*) untuk menurunkan logam tembaga (Cu) dalam limbah elektroplating studi kasus: industri kerajinan perak Kelurahan Citran, Kotagede. *Jurnal Presipitasi*. 7, 23-26.

Tan, T., Yu, J., Shang, F., 2011. Biorefinery engineering. *Comprehensive Biotechnology*. 2, 815-828. https://doi.org/10.1016/B978-0-08-088504-9.00138-0

Winters, C., Guéguen, C., Noble, A., 2017. Equilibrium and kinetic studies of Cu (II) and Ni (II) sorption on living *Euglena gracilis. Journal of Applied Phycology.* 29, 1391-1398. https://doi.org/10.1007/s10811-016-1040-z

Yusuf, D.M., 2014. Pertumbuhan populasi mikroalga *Spirulina platensis* (Geitler) pada konsenterasi logam berat tembaga (Cu). *Jurnal Biologi*. 3, 1-9.

Zhou, G. J., Peng, F.Q., Zhang, L.J., Ying, G.G., 2012. Biosorption of zinc and copper from aqueous solutions by two freshwater green microalgae *Chlorella pyrenoidosa* and *Scenedesmus obliquus. Environmental Science and Pollution Research*. 19, 2918-2929. https://doi.org/10.1007/s11356-012-0800-9