Asian Journal of Water, Environment and Pollution, Vol. 17, No. 3 (2020), pp. 13-17.
DOI 10.3233/AJW200029

Effect of Mercury on Growth of Several Microalgae

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Received February 2, 2020; revised and accepted June 5, 2020

Abstract: This study aimed to analyse the effect of toxic heavy metal on microalgae growth. Several microalgae i.e cyanophyceae (*Spirulina maxima*), eustigmatophyceae (*Nannochloropsis oculata*), chlorophyceae (*Chlorella vulgaris*) and porphyridiophyceae (*Porphyridium cruentum*) were exposed to mercury with various concentrations (1, 3 and 5 mg. L^{-1}). An experimental method was carried out in the laboratory scale with one control of microalgae culture without mercury exposure. The microalgae cultivated by using Walne medium with the initial cells were 10,000 cells.mL^{-1} for *S. maxima* and *N. oculata* respectively and 100,000 cells.mL^{-1} for *C. vulgaris* and *P. cruentum* respectively. The microalgae density was counted every day for 8 days. Water quality parameters were temperature, salinity, light intensity and pH. All treatments were set and calculated by using completely randomized design Anova and SPSS.16, respectively. The result depicts that the cell density of microalgae decreased during the culture except in control. A significant decay was shown by microalgae on exposure to 3 mg.L^{-1} of mercury, where final density of microalgae on the 8th day was 2,333 cells.mL^{-1} (*S. maxima*), 13,000 cells.mL^{-1} (*N. oculata*), 6,000 cells.mL^{-1} (*C. vulgaris*) and 2,000 cells.mL^{-1} (*P. cruentum*). However, all the quality indices are belonging to optimum condition for algae growth, such as temperature was 25-28°C, salinity was 34-38 ppt, light intensity was approximately 3,600 lux, and pH was 7-8. This research recommends to investigate the effect of heavy metals on ultrastructure of microalgae by using transmission electron microscope (TEM) and the chlorophyll content of microalgae contaminated.

Key words: Heavy metal, microalgae, pollution, toxic.

Introduction

Water pollution is characterized by the change in the physical, chemical and biological properties of water so that the quality of the environment becomes less or no longer functioning in accordance with its designation (Akbar et al., 2014; Dwivedi, 2017). Heavy metals are high molecular weight metal elements. Some types of heavy metals that often caused pollution is mercury (Hg), lead (Pb), chrome (Cr), cadmium (Cd) and arsenic (As). The presence of heavy metals in the environment can be caused due to disposal of waste containing of heavy metals without wastewater management (Caroline and Moa, 2015).

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Mercury (Hg) is the only metal that is liquid at room temperature. The colour is silvery white, odourless and can evaporate at 375°C. Mercury is commonly used as a colouring agent, cosmetics, paint industry, insect repellent and others. The impact of mercury contamination in living organism cause harm such as disturbance on nervous system, DNA and chromosome damage, skin allergies, miscarriage, disability or even lethal effect (Agustina, 2014; Soegianto et al. 2010).

Microalgae have been used as a remediation agent to treat water pollution. Some researches proved that microalgae are efficient to remove nutrient and heavy metals. Nutrient removal and heavy metals removal in water, associated with the growth of microalgae *N. oculata, S. maxima, C. vulgaris* and *P. cruentum* are some potential microalgae to be used as a remediation agent with low production cost (Jais et al*.,* 2017). However, in such condition microalgae will be affected negatively because of the higher concentration of heavy metals. Several microalgae were exposed on heavy metal and they show a decrease in growth such as an inhibitory effect on *Tetraselmis marina*'s growth by ion Co^{2+} and Ni^{2+} (Cameron et al, 2018), Cadmium decomposes polyphosphate bodies of *Chlamydomonas acidophila* culture (Nishikawa et al, 2003), lead (Pb) in *Chlorella sorokiniana* results in misshaped chloroplasts and formation of colonies of Chlorella cells possessing cytoplasm lipid droplets (Carfagna et al, 2013); therefore, the combination effect of aluminum (Al) and Pb on *Dunaliella tertiolecta* cause lysis on cell membrane (Sacan et al, 2007). The purpose of this study was to analyse the effect of toxic heavy metal mercury (Hg) on several microalgae growth such as *S. maxima*, *N. oculata*, *C. vulgaris* and *P. cruentum*.

Materials and Methods

Microalgae Preparation

The microalgae that are used in this study are *S. maxima, N. oculata, C. vulgaris* and *P. cruentum.* These microalgae strain was obtained from Situbondo BBPBAP, East Java. Each microalga was cultivated in Walne medium which contains 2.5 ml Walne, 2.5 ml vitamin, 2.5 L sterilized sea water and 250 ml microalgae for 3-4 days for acclimation before ready to run the experiment.

Heavy Metal Stock

One of the heavy metal compounds used in this study is $HgCl_2$. About 1.353 mg of mercury compound ($HgCl_2$) was diluted with 1 L of sterilised water to get different concentrations. The concentrations used in this study were 1 $mg.L^{-1}$, 3 $mg.L^{-1}$ and 5 $mg.L^{-1}$ of mercury.

Experimental Setup

This study was carried out experimentally with a complete randomised design consisting of three different concentrations (1, 3 and 5 $mg.L^{-1}$) and one positive control (microalgae without heavy metals), each with three replications. The initial cell density of microalgae used in this study was 10,000 $cells.mL^{-1}$ for *S. Maxima* and *N. oculata* and 100,000 $cells.mL^{-1}$ for *C. vulgaris* and *P. cruentum* during 8 days of culture. The biomass density was calculated using this equation (Borowitzka and Moheimani, 2013):

$$\text{Density}\left(\frac{\text{cell}}{\text{ml}}\right) = \frac{\text{total cell counted}}{\text{number of squares counted}} \times \text{volume} \times 10000$$

Each microalgae cell density was measured every day during 8 days of study to analyse the toxic effects of mercury on algae growth. Since water quality affects the growth of microalgae, all water quality such as temperature, salinity, light intensity and pH were maintained at optimum level for microalgae. Temperature was maintained at 25-28°C (DO meter Lutron PDO 519), salinity was 34-38 ppt, light intensity was approximately 3600 lux and pH was 7-8. Metal concentrations were determined by *Atomic Absorption Spectrophotometry.* Tests of between-subjects' effects were performed with SPSS 16.0 software.

Growth Rate and Generation Time

Growth rate and generation time were calculated as following equation (1) and (2), severally (Prasetiya, 2016 and Windarto, 2014).

$$x = \frac{\ln N2 - \ln N1}{d2 - d1} \quad (1)$$

where x (day^{-1}) is growth rate, $N1$ and $N2$ representing the cell density at the start and the end of each growth period, while $d1$ and $d2$ show the measurement time.

$$G = \frac{t \log 2}{\log b - \log B} \quad (2)$$

where G is generation time (time $generation^{-1}$), t is culture time, b and B are concentration of microalgae at the end and at the beginning of the observation, correspondingly.

Results and Discussion

Microalgae density during the study was counted every day during study. All the density of all treatments was measured for density both by adding heavy metals and without adding heavy metals. Data on the average density of microalgae can be seen in Figure 1.

In the control treatment, all microalgae populations tended to follow the growth phase pattern and experienced an adaptation phase, which was marked by changes in the number of cells that began to increase on the day 1. Phase lag or adaptation phase is a phase that begins with cell adaptation to the new environment. This adaptation phase of cell density has not changed, but the cell size in that phase has increased (Leksono et al., 2017).

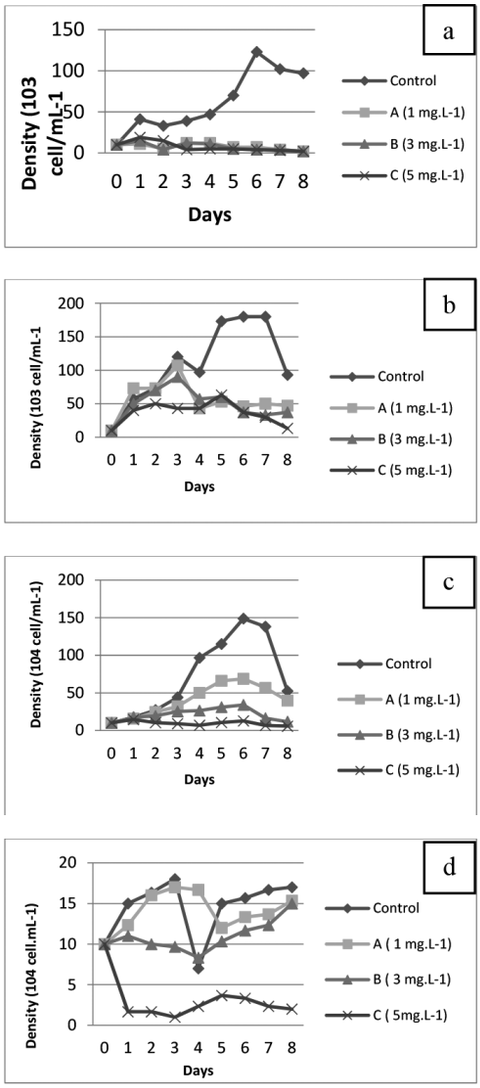

Figure 1: (a) *S. maxima*, (b) *N. oculata*, (c) *C. vulgaris* and (d) *P. cruentum*.

The exponential phase in all microalgae populations occurred on day 6 of the control treatment and had the highest density compared to other treatments. This is presumably due to the influence of heavy metal mercury exposed to the culture medium. The density of microalgae is, respectively, *S. maxima* 123×10^3 cell.mL^{-1}, *N. oculata* 180×10^3 cell.mL^{-1}, *C. vulgaris* 149×10^4 cell.mL^{-1} and *P. cuentrum* 16×10^4 cell.mL^{-1}. While the stationary phase in the control treatment, the population of microalgae decreased (*S. maxima*, *N. oculata* and *C. vulgaris*) on the 7th day except *P. cruentum* which has increased. This is presumably because the supply of nutrients in the *P. Cruentum* culture media is still able to support the growth of the algae. The higher the availability of nutrients in the culture media, they will be able to increase growth in microalgae (Blair et al., 2014).

Based on the result of the Mann Whitney analysis test using SPSS regarding the effect of heavy metal mercury (Hg) on the growth of microalgae (*Spirulina maxima*, *N. oculata, C. vulgaris* and *P. cuentrum*) in culture media sig (0.000) < (0.05) indicates that H_0 is rejected and H_1 is accepted. It means that the addition of heavy metal mercury (Hg) in culture media can influence the growth performance of microalgae. This is presumably because the cell adsorbs the heavy metal content of mercury (Hg) in culture media.

Simanjuntak et al. (2016) reported that the decrease in cell numbers was caused by a Hg compound ($HgCl_2$). The Hg compound is a heavy metal toxic on microalgae growth and can inhibit cellular growth if the amount given is excessive of their tolerance concentration. The higher concentration of heavy metals given, it will inhibit the growth, photosynthesis and inhibit fat formation in microalgae (Wang et al., 2012). The inhibition of photosynthesis is caused by the destruction of pigments in chloroplast which functions to absorb light and inhibits enzymes (nitrate reductase and alkaline phosphatase) involved in CO_2 fixation (Afkar et al., 2010). In addition, decreasing chlorophyll induced by heavy metals can be caused by inhibition of chlorophyll synthesis and enzyme work systems. Chlorophyll damage will cause the inhibition of photosynthesis process then it can influence the growth of microalgae (Maznah et al., 2011). The final density of microalgae on the eighth day were *S. maxima* 2,333 cell.mL^{-1}, *N. oculata* 13,000 cell.mL^{-1}, *C. vulgaris* 6,000 cell.mL^{-1} and *P. cruentum* 2,000 cell.mL^{-1}.

Results for each treated microalgae have different growth rates, this is due to differences in the types of microalgae, nutrient conditions and the environment.

This determines the time needed by microalgae to reach the maximum growth phase or can be called exponential (Prayitno, 2016).

Table 1: Microalgae growth rate

Growth rate (Day^{-1})	*A*	*B*	*C*	*K*
S. maxima	-0.179	-0.179	-0.179	0.252
N. oculata	0.193	0.164	0.033	0.279
C. vulgaris	0.172	0.019	-0.071	0.207
P. cruentum	0.053	0.051	-0.201	0.066

A – 1 mg.L^{-1}, *B* – 3 mg.L^{-1}, C – 5 mg.L^{-1} and K – Control 0 mg.L^{-1}.

The maximum growth rate in *S. maxima* is in the control treatment (K) with a yield of 0.252 day^{-1}, while the minimum growth is in all three treatments namely A, B and C because it has the same value of –0.179 day^{-1}. In the *N. oculata* microalgae, the maximum growth results were in the Control (K) treatment with a value of 0.279 day^{-1}, while the minimum growth was in C treatment with a value of 0.033 day^{-1}. Furthermore, the results of the growth rate in the *C. vulgaris* microalgae obtained maximum growth in the control treatment (K) with a value of 0.207 day^{-1}, while for the minimum growth rate there was in treatment C with a value of –0.071 day^{-1}. In *P. cruentum,* maximum growth results are in the control treatment with a value of 0.066 day^{-1}, while the minimum growth rate is in treatment C with a value of –0.201 day^{-1}. To sum up, it can be concluded that the best growth rate is in the control treatment because there is no heavy metal mercury exposure. Otherwise, the growth rate occurs slowly in treatment C with a concentration of mercury heavy metals of 5 mg. L^{-1}. Heavy metal content of 5 mg. L^{-1} is included in the category of highly toxic or toxic depending on the *t* level at which microalgae cannot grow properly.

Acknowledgement

This research was conducted by financial support from HPP (*Hibah Peneliti Pemula*) Grant 2019 provided by LPPM of Universitas Brawijaya.

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Asian Journal of Water, Environment and Pollution

Volume 16 Number 3 **July – September, 2019**

Contents