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THE GROWTH BEHAVIOR OF FRESHWATER AND MARINE MICROALGAE EXPOSED TO CD AND CU

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ABSTRACT

Cadmium and copper are essential and non-essential heavy metals that often appear as water pollutants as a residue of land-based activity. Both of these metals can disrupt metabolism and inhibit the growth of Microalgae. This study is one of continued-research of our long-term study to conclude the life history of both freshwater and marine microalgae living in heavy metal-stress environment. The heavy metal exposed test was conducted in 3 species of microalgae namely *Chaetoceros calcitrans*, *Skeletonema costatum*, and *Nannochloropis* sp. The main goal of this research is to find out their growth-behavior under the presence of Cd and Cu. The concentrations used were 0.7, 1.3, and 1.9 ppm with 3 replications of each. Exposure carried out for 96 hours using microalgae aged 4 days (on their exponential phase). Calculation of microalgae density is conducted manually every 12 hours to monitor their growth response to particular concentration.

KEY WORDS : Cadmium, Copper, Environment stress, Inhibitor, Microalgae

INTRODUCTION

Technology and science are developing rapidly nowadays, however, it causes both positive and negative impacts on humans. One negative impact that deserves attention is environmental pollution due to industrial progress. In addition, household activities also contribute to environmental pollution (Pratiwi *et al.*, 2019). Various wastes are produced by anthropogenic activities, one of which is heavy metals. Industrial activities often create wastewater containing heavy metals that flow into natural waters (Lee, 2011). Heavy metal is a type of pollutant that is persistent in the environment and its presence in water can accumulate in sediments and biota (Hawkins, 2017). Examples of heavy metals that are often found in aquatic environments include cadmium and copper. Heavy metals Cd and Cu have phytotoxic characteristics when in an aquatic environment with high concentrations (Nassiri *et al.*, 1997). Excessive presence of heavy metals in waters can change their properties to be toxic. This applies to both essential and non-essential metals. The presence of heavy metals in the environment can affect many things in the food chain cycle, such as inhibiting cell growth and development, inhibiting reproduction, causing cell morphological changes, and disrupting cell metabolism (Cid *et al.*, 1995).

Primary productivity in waters is influenced by the presence of phytoplankton, where they are dominated by microalgae that play an important role in the balance of the marine ecosystem. Microalgae are also at the basic level of the food chain, so they will have the greatest effect due to the toxicity of heavy metals in the environment

(Zeitzchel, 1978). Copper is an essential type of metal, a metal that is needed by microalgae for its metabolism in very small amounts. In large concentrations, copper will be phytotoxic and can inhibit the growth of microalgae and cause death (Yan and Pan, 2002). Whereas cadmium is a bivalent heavy metal that is very toxic with a widespread in waters (Templeton and Liu, 2010). Cadmium can have a negative impact on microalgae growth, including growth inhibition, suppression of mitotic activity and cell division, disruption of cell wall composition and disintegration of chloroplast structures (Vecchia *et al.*, 2005). Based on this, this study aims to determine the growth response of microalgae *Chaetoceros calcitrans, Skeletonema costatum*, and *Nannochloropis* sp. against cadmium and copper exposure.

MATERIALS AND METHODS

Chaetoceros calcitrans, Skeletonema costatum and *Nannochloropis* sp. used in this study were obtained from pure cultures selectively in the laboratory and acclimatized to ensure that microalgae were free of other pollutants. At the time of exposure, the microalgae used were 4 days old or in an exponential phase. The media used during the exposure process are sterilized seawater. At the beginning of the exposure, microalgae were given vitamin, silicate, and diatomic fertilizer for *Chaaloceros calcitrans* and *Skeletonema costatum*, while for *Nannochloropis* sp. given Conwy fertilizer and vitamin B12. All equipment used were sterilized using an autoclave.

Cd and Cu heavy metals are diluted using sterile seawater to have concentrations of 0.7, 1.3, and 1.9 mg/L. The solution was added to the 500 mL Erlenmeyer flask then microalgae and nutrients were added. Each microalga and concentration test was performed 3 times. Exposure to heavy metals carried out for 96 hours and during the process of exposure to microalgae was given aeration and good lighting to supply the needs of photosynthesis. Microalgae density was calculated to determine the response of microalgae to heavy metal exposure. Calculation of density is calculated once every 12 hours using a microscope and haemocytometer.

RESULTS AND DISSCUSSION

The density of *Chaetoceros calcitrans, Skeletonema costatum*, and *Nannochloropsis* sp. decreased density in all test concentrations, this can be seen in the percentage value of dead microalgae in Table 1.

The number of dead populations have increased with increasing heavy metals test concentrations. This happens to all types of microalgae. Trendline of the increase in the percentage of microalgae deaths in cadmium treatments can be seen in Figure 1.

The decreasing rate of microalgae on cadmium exposure showed an increase with increasing test concentrations. Based on this it shows that cadmium exposure can influence cell growth from the initial exposure. The same thing was found by Thomas *et al.* (1980) that cadmium can reduce the level of microalgae density significantly. The percentage of microalgae mortality on Cu exposure also shows the same trendline as Cd exposure Figure 2). Population

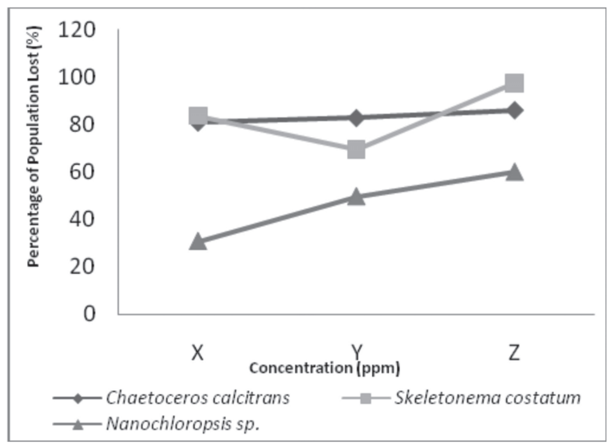

Fig. 1. Trendline of Population Lost on Cadmium Treatment

Table 1. Decreasing of microalgae community during heavy metals exposure.

Concentration (ppm)	Population Lost (%)					
	Chaetoceros calcitrans		*Skeletonema costatum*		*Nannochloropsis* sp.	
	Cd	Cu	Cd	Cu	Cd	Cu
X	81	87.4	83.7	22.68	30.7	38.53
Y	82.9	91.8	69.5	41.76	49.8	59.4
Z	86.2	97.1	97.5	36.68	60.2	71.7

(Notes: X = 0.7 ppm; Y = 1.3 ppm; Z = 1.9 ppm) lost has increased with increasing concentrations of heavy metals. This is also in accordance with previous studies, namely the level of microalgae growth inhibition will increase in the higher exposure to heavy metals (Markina and Aizdaicher, 2006).

The percentage of mortality in Cu exposure also increased along with the increased concentration of exposure. *Chaetoceros calcitrans* and *Nannochloropsis* sp. the percentage of mortality in Cu exposure indicates a higher value than Cd exposure. Whereas in *Skeletonema costatum* the mortality in cadmium treatment is greater than copper. That is because the resistance of microalgae to pollutants varies. In addition, the death rate of microalgae is also influenced by the ability of microalgae to absorb heavy metals from the environment. The absorbance process of heavy metals into microalgae cells is influenced by several things, including the characteristics of microalgae cell walls, the concentration of heavy metal ions in the environment, the amount of microalgae biomass and chemical and physical parameters such as temperature, pH, DO, etc (Aksu, 2006).

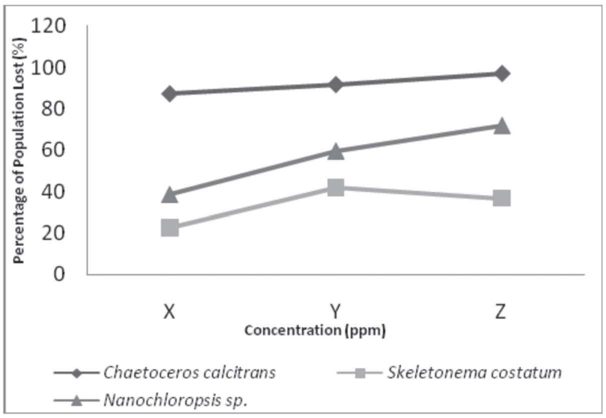

Fig. 2. Trendline of Population Lost on Copper Treatment

Another factor that causes differences in the death rate of microalgae is the characteristics of heavy metals. Cadmium and copper are heavy metals with different characteristics. Copper is an essential heavy metal that is needed as a nutrient of microalgae with very small amounts, while high copper concentrations in the environment will cause copper to be toxic. In addition, copper also has the property of being soluble in fat (lipophilic) so that copper will be more easily absorbed into microalgae cells. This causes a higher rate of microalgae mortality (Toncheva-Panova *et al.*, 2006).

Unlike copper, cadmium has lipophobic properties or is difficult to dissolve in fat, to be able to enter the microalgae cell membrane, cadmium takes more time than copper. This process also requires facilitated diffusion events. In addition, the content of cadmium in water can cause significant inhibition of the photosynthetic process of microalgae. When viewed morphologically, cadmium can cause swelling in the mitochondria of cells which results in cellular disruption (Trevor *et al.*, 1986). Therefore, the mortality rate of microalgae *Chaetoceroscalcitrans* and *Nannochloropsis* sp. on cadmium exposure shows lower values when compared to copper.

Chaetoceros calcitrans and *Skeletonema costatum* are diatomic microalgae which consist of carboxylic, hydroxyl, amino, sulfhydryl, sulfate and phosphate groups (Moreno-Garrido *et al.*, 2000). Heavy metal contamination in the environment can be toxic to diatom microalgae. High heavy metal concentrations in the waters can reduce the productivity of diversity and changes in diatomic species communities (Sabater, 2000). High concentrations of heavy metals in the media can also cause morphological changes and decreased growth rates (Morin *et al.*, 2007).

On cadmium exposure, *Nannochloropsis* sp. shows the smallest percentage of mortality compared to 2 other microalgae. Nannochloropsis sp. is a green alga that has a smaller size compared to other microalgae. This type of microalgae has the ability to absorb heavy metals and is an efficient microalga in handling waters that are polluted by heavy metals (Rose *et al.*, 2018). Walls of Nannochloropsis sp. composed of strong cellulose components and complex carbohydrates that are useful for binding toxic substances and regulating the activity of the immune system (Bold and Wynne, 1985).

CONCLUSION

High levels of heavy metals in the environment will have a negative impact on the ecosystem. Heavy metals that often appear in the liquid include cadmium and copper. Copper is an essential heavy metal, but if the concentration in the waters is excessive it will be phytotoxic. While cadmium is a non-essential heavy metal so that its presence in the environment will be a pollutant for the ecosystem. In this study, cadmium, and copper both can cause significant death of microalgae. Reduction in density between microalgae varies with each exposure. This is because the composition of cell walls between microalgae is different, so that each microalga has a different resistance to pollutants in their environment.

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