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# Microalgae *Skeletonema costatum* for Cd and Cu Remediation

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**Abstract**. Cadmium (Cd) and copper (Cu) are types of heavy metals that can have an adverse effect on the ecosystem. Even copper is an essential metal but in limited concentrations, however, it leads to a toxic effect on the environment when used in high concentrations. Bioremediation of these metals can be done using microalgae *Skeletonema costatum*. In this study, bioremediation tests of Cd and Cu were carried out on a laboratory scale using various concentrations, control, 0.7, 1.3 and 1.9 ppm, respectively. The metals exposure was carried out for 96 hours. During the test the microalgae population was monitored, and the metal concentration in organism was calculated. The final analysis is to determine bioaccumulation factors as indicators of microalgae ability as heavy metals bioremediation-agent. Cd inhibits the growth of microalgae which is more than Cu. Yet, Cu is easily absorbed by *Skeletonema costatum* than Cd. The final analysis is to determine bioaccumulation factors as indicators of microalgae ability to be heavy metals bioremediation-agent in the bioconcentration factors (BCF) of Cd and Cu average are 6.15 and 7.97, respectively.

**Key words**: Bioaccumulation, bioremediation, heavy metal, microalgae.

## Introduction

Cadmium is a bivalent type of heavy metal that has highly toxic properties and is wide spread in soil and aquatic environment caused by anthropogenic activities (Guntur et al., 2019; Putranto et al., 2014). Cadmium pollution arises specifically from increased emissions from electronic components in industrialised countries. This is a serious threat to human health because it enters into the food chain through accumulation by plants (Templeton and Liu, 2010). Cadmium absorption in both land and marine plants are influenced by high water solubility, chemical properties and physical similarity with essential cations such as $Fe^{2+}$, $Cu^{2+}$, Zn and $Ca^{2+}$ enabling the utilisation of transporters for Cd absorption (Daud et al., 2009). In plants, cadmium has adverse effects such as suppression of mitotic activity and cell division, inhibition of growth, the disintegration of the structure of chloroplasts and disruption of cell wall composition (Vecchia et al., 2005). In addition, cadmium also inhibits the absorption of important nutrients from these plants, interferes with photosynthetic activity, and

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binds SH groups from essential enzymes and proteins (Andosch et al., 2012).

Copper is an essential nutrient needed in small quantities (5-20 µg/g) by humans, other mammals, fish and shellfish for carbohydrate metabolism and has functions when combines with more than 30 enzymes. In addition, Cu is also needed for the formation of haemoglobin, haemocyanin and oxygen-carrying pigments invertebrates and shellfish (Soegianto et al., 2013; Usman et al., 2013). However, copper concentrations exceeding 20 µg/g cause toxic effects on organisms (Bradl, 2005). Copper is widely used as a mixture of metals, equipment, coins, jewellery, electrical cables, car brake pads, armour and others. In high concentrations, copper is a highly toxic metal and can kill algae, fungi and mollusks. Copper has characteristics that are easily soluble in water and bind to sediments and organic materials (Solomon, 2009). This causes copper to easily accumulate in the organism's body and enter the food chain (Pratiwi et al., 2019).

Two types of metal mentioned above are heavy metals that are often found in aquatic environments. If its existence in the environment is allowed to increase continuously, it will have a negative impact on the sustainability of the ecosystem. Therefore, environment friendly technology is needed which can reduce its presence in the environment. Some techniques can be used to remove heavy metals from the environment, including electrodialysis, precipitation and reverse osmosis, but some of these techniques are expensive and difficult to do for concentrations of heavy metals below 100 mg/L. Bioremediation using microorganism cells can play an important role in the management of wastewater contaminated with heavy metals. This technique is cheap and affordable to remove heavy metals in water with concentrations below 100 mg/L.

Biomass of microorganisms can absorb heavy metal ions from water media even when cells are killed (Rangsayatorn et al., 2002). One of the microalgae that can be used as a heavy metal bioremediation agent is *Skeletonema costatum*, this type of diatom is able to absorb heavy metals in two ways, namely absorbing and adsorbing. The purpose of this study was to monitor the growth-behaviour of *Skeletonema costatum* exposed to heavy metals and to determine the ability of it as bioremediation-agent.

## Materials and Methods

The media used in this study is sterilised seawater. Cadmium and copper heavy metal pollutants are derived after dilution of stock solutions (1000 ppm) to concentrations of 0.7, 1.3 and 1.9 ppm. *Skeletonema costatum* microalgae used in this study originated from the pure culture in a 4-day-old culture laboratory, this is because at this stage *Skeletonema costatum* is in an exponential phase. Before exposure, microalgae are acclimatized and ensured to be free of pollutants. On exposure, microalgae give nutrients needed in the form of vitamins, silicates and diatom fertilizers.

The study was conducted using Cd and Cu with concentrations of each control, 0.7, 1.3 and 1.9 ppm. Each concentration was tested with three replications. The testing process was carried out using 500 mL Erlenmeyer and giving proper aeration and good lighting conditions for photosynthetic purpose. The research was conducted in a sterile and pollutants free laboratory. The time of heavy metal exposure was carried out for 96 hours and every 12 hours microalgae density was calculated to determine the growth response of microalgae on exposure to heavy metals. This calculation is done using a microscope and a haemocytometer. In addition, environmental parameters are also monitored every day to ensure that the media is in good condition. At the end of the exposure, heavy metal concentrations in the media and microalgae were calculated. The results of this calculation are used to determine the bioconcentration factors (BCF) value or value used to determine how much *Skeletonema costatum* is capable of absorbing heavy metals from the environment.

## Result and Discussion

The results of the measurement of *Skeletonema costatum* microalgae every 12 hours can be seen in Table 1 and Table 2.

After Cd exposure, *Skeletonema costatum* microalgae showed a decrease in density response which increased with increasing exposure time. Even in the second measurement, at the time of sampling 12 hours exposure, the decrease in microalgae cells occurred in almost 50% of the initial population. This is because the characteristics of cadmium are toxic to microalgae. The influence given by Cd heavy metals increases with increasing concentration. The same results were also obtained by Thomas et al. (1980) that heavy metals Cd can reduce the level of cell density of *Chaetoceros* sp. significantly. Addition of cadmium concentration in the media also causes decreased chlorophyll-A content (Reinfelder et al., 2000), which can result in

**Table 1: *Skeletonema costatum* cell density at exposure to Cd**

| *Time (hours)* | *Growth of microalgae (x $10^4$ cell/mL)* | | | |
|---|---|---|---|---|
| | *O* | *X* | *Y* | *Z* |
| 0 | 24.67±2.75 | 24.50±0.50 | 25.17±0.58 | 24.33±0.76 |
| 12 | 25.83±1.04 | 16.67±0.76 | 13.67±0.76 | 12.67±0.76 |
| 24 | 28.50±1.50 | 13.17±1.76 | 10.50±0.50 | 11.00±0.50 |
| 36 | 25.83±1.44 | 10.33±1.04 | 11.50±0.50 | 4.83±0.76 |
| 48 | 32.83±3.79 | 10.17±1.26 | 9.50±0.50 | 5.50±0.87 |
| 60 | 29.33±0.76 | 8.67±0.58 | 6.50±0.50 | 1.50±0.50 |
| 72 | 33.33±2.52 | 6.50±1.00 | 7.33±0.76 | 2.17±0.76 |
| 84 | 34.83±1.04 | 6.17±1.04 | 8.83±0.76 | 1.67±0.76 |
| 96 | 35.33±0.76 | 4.00±1.00 | 7.67±0.58 | 0.67±0.76 |

Notes: ± – STDEV; O – Control; X – 0.7 ppm; Y – 1.3 ppm; Z – 1.9 ppm.

**Table 2: *Skeletonema costatum* cell density at exposure to Cu**

| *Time (hours)* | *Growth of microalgae (x $10^4$ cell/mL)* | | | |
|---|---|---|---|---|
| | *O±STDEV* | *X±STDEV* | *Y±STDEV* | *Z±STDEV* |
| 0 | 25.50±1.32 | 25.00±1.04 | 24.33±0.76 | 25.00±0.50 |
| 12 | 26.00±1.04 | 24.33±0.29 | 20.33±0.29 | 20.33±1.04 |
| 24 | 29.00±1.15 | 22.00±1.76 | 18.83±1.15 | 19.00±1.00 |
| 36 | 25.83±1.00 | 24.00±1.00 | 17.33±0.29 | 18.00±0.50 |
| 48 | 27.33±1.04 | 22.67±0.29 | 17.33±0.29 | 17.33±0.58 |
| 60 | 29.67±0.76 | 20.83±0.29 | 15.00±0.50 | 15.33±0.29 |
| 72 | 31.00±0.50 | 20.00±1.00 | 16.00±0.87 | 16.17±0.76 |
| 84 | 34.17±0.58 | 18.50±0.87 | 14.17±0.76 | 15.83±0.76 |
| 96 | 33.67±1.04 | 19.33±0.29 | 14.17±0.58 | 15.83±0.76 |

Notes: ± – STDEV; O – Control; X – 0.7 ppm; Y – 1.3 ppm; Z – 1.9 ppm.

a decrease in photosynthesis and nutrient absorption (Wang and Dei, 2001). The final results of cell density measurements were tested statistically to determine whether cell decline occurred due to exposure to heavy metals and the results showed that exposure to heavy metals reduced cell density specifically. This is also supported by the results of cell density calculations in the control exposure that continue to increase during the testing process.

The results of the calculation of the microalgae density of *Skeletonema costatum* on Cu exposure showed a more stable decline compared to Cd exposure. Copper is an essential heavy metal that functions as a micronutrient for living things. Cu is needed by organisms for their biological needs in a very small amount so that in high concentrations in the Cu environment can reduce cell density greater with increasing exposure time. This is also in accordance with previous studies, namely the rate of inhibition of microalgae growth will increase in exposure to increasingly heavy metals (Markina and Aizdaicher, 2006).

According to Baron et al. (1995), some heavy metals play an important role in living organisms at very low concentrations. In small quantities, essential metals help in growth, metabolism and enzyme activity, but at high concentrations, they are toxic and have a direct influence on physiological, biochemical and growth-inhibition processes. Therefore, *Skeletonema costatum* has decreased density on exposure to cadmium and copper.

At the end of exposure, at 96 hours, heavy metal levels were measured in the media and microalgae using atomic absorption spectrophotometry (AAS). Media and microalgae are separated by using Whatman filter paper number 5. The results of these measurements can be seen in Table 3.

**Table 3: Measurement of heavy metals concentration**

| | *Treatment (ppm)* | *Water (ppm)* | *Body (mg/kg)* |
|---|---|---|---|
| Cd | 0.7 | 0.58 | 6.22 |
| | 1.3 | 1.15 | 5.63 |
| | 1.9 | 1.80 | 5.12 |
| Cu | 0.7 | 0.55 | 7.20 |
| | 1.3 | 1.11 | 7.91 |
| | 1.9 | 1.77 | 6.72 |

The concentration of heavy metals Cd and Cu in water decreased at the end of the exposure. The level of decrease in copper concentration in the media is observed to greater than cadmium. However, the rate of decline did not show a big difference. Photosynthetic microorganisms such as microalgae are relatively easy to treat wastewater in a growth medium and even have the ability to carry out absorption with high capacity and selectivity for the absorption of strong metal ions (De Philippis et al., 2011; Markou et al., 2015; Micheletti et al., 2008; Rossi et al., 2012).

Some microalgae and cyanobacteria are tolerant and highly resistant to metal toxicity (Bux and Chisti, 2016). Metal ions in the environment can be degraded through two stages, namely the adsorption and bioaccumulation processes. Initially, metal ions are passively adsorbed on the cell surface (on live and non-living biomass), this process only occurs in a few seconds or minutes and then the ion is transported slowly in the membrane cell and some are accumulated intracellularly. Bioaccumulation only occurs in cells, metal ions move across cell membranes and then bind to cytoplasmic proteins or polysaccharides (Dwivedi, 2012; Gupta and Rastogi, 2008; Kumar et al., 2015). It is shown that the concentration of heavy metals in the media decreases, yet increases in microalgae cells at the end of the exposure.

The results of the calculation of the absorbance of heavy metals are used to determine the potential of *Skeletonema costatum* in carrying out the accumulation of Cd and Cu heavy metals through the BCF value. The BCF value can be seen in Figure 1.

Based on the value of BCF, this study showed that the ability of the accumulation of heavy metals of *Skeletonema costatum* microalgae will decrease in higher concentrations of heavy metals. This occurs in heavy metals Cadmium and Copper. BCF is the rate of absorption of a chemical carried out by an organism from the environment through respiration or cell surface. Therefore, BCF is used to determine the level of accumulation of a chemical compound under controlled laboratory experiments (Arnot and Gobas, 2006). The concentration of heavy metals in microalgae reflects the concentration of heavy metals in the environment. Research carried out on the Ugandan river shows that *P. cruentum* is a bioaccumulator with Cu> Cd levels (Sekabira et al., 2011). BCF values greater than 1 indicate that microalgae can be used as heavy metal bioaccumulators, but microalgae is considered as a good accumulator if the BCF value is greater than 1000 (Conti and Cecchetti, 2003). Based on the BCF values obtained from this study, it was shown that *Skeletonema costatum* can be used as a low-ability bioaccumulator of Cd and Cu heavy metals.

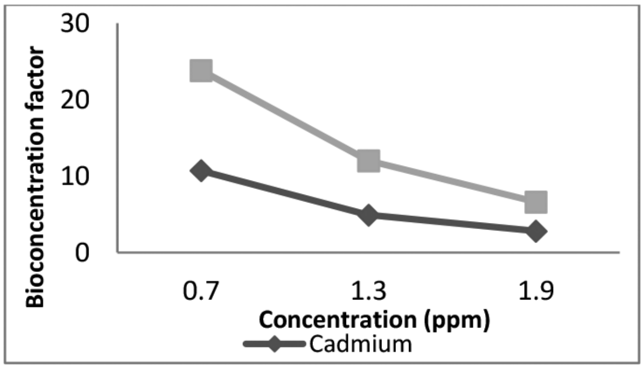


**Figure 1: Bioconcentration factors of *Skeletonema costatum.***

## Conclusion

Cadmium is a heavy metal that has toxic properties and its presence in the environment through anthropogenic activities. While copper is an essential type of heavy metal that is needed by organisms for their life needs in very low concentrations. However, the existence of these increases due to land-based disposal input to the ocean. This will potentially cause disturbing the food-chain. Bioremediation apparently can be applied to this situation using *Skeletonema costatum* as a heavy metals-bioaccumulation agent.

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