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COMBINATION OF SEAWEED *(GRACILARIA SP.)* AND BLOOD COCKLES (*ANADARA GRANOSA*) WITH ZEOLITE AS BIOFILTER TO REDUCE LEAD (PB) IN NPK FERTILIZER WASTE

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ABSTRACT

The biofilter is a wastewater treatment system that is carried out by flowing wastewater into a biological reactor filled with a filter to reproduce contaminant decomposer microorganisms that contained in wastewater either using aeration or without aeration. This study objective is to determine the appropriate combination to reduce lead (Pb). This study uses an experimental method with a completely randomized design consisting of five treatments group and four replications each group. The treatment group in this study are proportion differences of Gracillaria sp. and Anadara granosa combination. The parameters observed in this study were the levels of heavy metals in seaweed, cockles, and water. Data analysis using Analysis of Variant (ANOVA) and continued with Duncan's Multiple Range Test. The results show that the use of a combination of Gracillaria sp. and Anadara granosa can absorb lead (Pb) in NPK fertilizer waste with the best combination in P2 treatment group with a proportion of 50% Seaweed (Gracillaria sp.), 50% Blood cockles (Anadara granosa) and Zeolite. The combination of Gracillaria sp. and Anadara granosa as biofilter can be used as an alternative in a wastewater treatment system to reduce lead (Pb) pollution which is very dangerous for the environment.

Keywords: *Anadara granosa, Gracillaria sp.*, Biofilter combination, Wastewater treatment

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1. INTRODUCTION

Heavy metal pollution is very dangerous for the environment. Environmental pollution occurs because any discharge of organism, substances, energy and / or other components into the environment and / or changes in the environment by human activities or natural processes so that quality of environment decreases to a certain level which causes the environment to decrease in term of function or unable to function completely (Singh et al., 2011). While the definition of pollution according to Constitutional Law No. 32 of 2009, Pollution is any discharge of organism, substances, energy and/or other components into the environment by human activities, so that it exceeds the environmental quality standards.

One of the heavy metals that are in the waters and toxic to an organism is lead (Pb) (Tchounwou et al., 2012). The presence of Pb in water with high concentration can kill aquatic biota. Pb with 188 ppm concentration can kill fish, in crustaceans, the concentration of 2.75-49 ppm after 245 hours may lead to death (Handhani et al., 2017). According to Minister of Environment Decree number 51 of 2004, the criteria for seawater quality standards for Pb concentration for marine biota is 0.008 ppm. Acute Pb toxicity in the waters can cause damage to the kidneys, reproductive system, liver, brain, central nervous system, and may lead to death (Jaishankar et al., 2014). Given the dangers that can be caused by Pb to an organism, their existence in the environment, especially in the waters, must be minimized. One way to reduce Pb concentration in waters is to use biofilter, which is a method that is carried out by using live organisms which aims to reduce pollution or contaminant in an aquatic environment that contains toxic materials (Yulianto et al., 2006).

Generally, fuel oil gets additional tetraethyl substances containing Pb to improve quality so that the waste from these vessels can cause high levels of Pb in these waters and lead (Pb) can also originate from rusty pipes (Chowdhury et al., 2017; Rochyatun et al., 2010). Blood cockles are often used as indicators of heavy metal pollution because they are able to accumulate heavy metal from the environment, widely distributed, have a sedentary nature, and are filter feeder (Mostafa et al., 2009). Blood cockles can also accumulate metals from food (such as phytoplankton, small protozoa, and bacteria), water and sediments (Wang et al., 2010). These following blood cockles are commonly used as an indicator of heavy metal pollution include green mussels, blood cockles and feather oyster (Arifin et al., 2010; Ruangwises and Ruangwises, 2011; Zahir et al., 2011)

Gracillaria sp. is a macroalga that has high adaptability against the changes in water quality (Handhani et al., 2017). High adaptability to changes in water quality makes Gracillaria sp. able to survive in the stress of heavy metals without disrupting the growth rate. Gracillaria sp. also has a tolerance limit in accumulating heavy metals, if the levels of heavy metals in the talus are too high for a long time, the growth activity in Gracillaria sp. will decreased (Yulianto et al., 2006).

Zeolite is a porous material with a wide range of usage. The use of zeolite is based on its ability to carry out ion exchange, adsorption and act as a catalyst (Ginting et al., 2013). Activated zeolite can reduce or decrease the content of Fe, Mn, Zn, and Pb metals found in groundwater (Rahman and Hartono, 2004). Zeolites have a very high absorption capacity for toxic materials. The size and type of zeolite considerably affect the adsorption capacity of ions (Ginting et al., 2013). A biofilter is a wastewater treatment system that is carried out by flowing wastewater into a biological reactor equipped with a filter to reproduce contaminant decomposing microorganisms contained in wastewater either using aeration or without aeration (Filliazati, 2013).

Filters used in recirculation systems are used as a medium for a microorganism to attach, microorganism will use organic materials that contained in feed waste as energy.

Recirculation system uses a filter material that can be used as a double, where biological, physical and chemical processes can run through it. Materials that can be used as a double i.e: coral, zeolite, and clamshell, this material has a dual function during the oxidation process. This study aims to determine the combination of seaweed, blood cockles with zeolite as a biofilter, and which composition that suitable for lead (Pb) reserves.

2. MATERIALS AND EXPERIMENTAL PROCEDURES

2.1. Preparation and Rearing Conditions

Initial preparation is done before the aquarium is used, namely the aquarium is washed with clean water and dried. After the washing process, an aquarium is soaked with a chlorine solution of 150 mg / l for 12-24 hours and to remove chlorine odor and dirt, the aquarium is washed using detergent by washing the aquarium wall and aeration hose to kill parasites, then flush it using clean water (Prakosa et al., 2013). The aquarium was then filled with 10 liters of seawater with 25 ppt salinity and equipped with aerator, water pumps, nets, blood cockles, seaweed and zeolite in each aquarium.

The blood cockles that will be used in this study are obtained from fishermen, blood cockles are brought alive. Blood cockles that will be used as filters are acclimatized first before used as experimental material. The blood cockles used in this study were 600 g for 100% filters, 450 g for 75% filters, 300 g for 50% filters and 150 g for 25% filters. The density of blood shell used is 600 g with an aquarium size of 40 cm x 30 cm x 30 cm. Based on FAO 2008 standards regarding clam density that used for small scale is 500 liters with a maximum density of 30 kg.

The seaweed used in this study is Gracilaria sp., This species is obtained from ponds in Surabaya. Seaweed that is brought alive. Seaweed that will be used as a filter was acclimatized beforehand then used as experimental material. Seaweed used is 210g for filters 100%, 158g for filters 75%, 105g for filters 50%, 53g for filters 25%. Determination of seaweed-based on the volume of water used. According to previous research seaweed densities used for filters is 500gr in an aquarium 80cm x 60cm x 50 cm (Yulianto et al., 2006).

The combination of blood cockles (*Anadara granosa*) and Seaweed (*Gracilaria sp.*) With Zeolite as biofilter has the following proportion: P0 (100% Gracillaria sp.), P1 (75% Gracillaria sp. 25% *Anadara granosa*), P2 (50% Gracillaria sp., 50% *Anadara granosa*), P3 (25% Gracillaria sp., 75% *Anadara granosa*) and P4 (100% *Anadara granosa*). This study uses five aquariums, each aquarium consisting of a combination of blood cockles, seaweed, and zeolite. Then the treatment is done for 7 days and water quality is checked every day.

2.2. Study Parameter

The parameters measured in this study are including the main parameters and supporting parameters. The main parameter, which is a decrease in the lead (Pb) level. Supporting parameters include water quality, as follow, temperature, dissolved oxygen (DO), nitrate, nitrite, ammonia, salinity. The main parameter analysis is by examining the Pb metal level in blood cockles, seaweed and water quality at the end of the experiment. Supporting parameter analysis using a thermometer, refractometer, pH pen, dissolved oxygen test kit, ammonia test kit, nitrite nitrate test kit, and spectrophotometer.

2.3. Biofilter Combination Maintenance

The blood cockles used in this study were blood cockles from Sedati, Sidoarjo city weighing 7-8 grams/clam. The blood cockles are cleaned first then acclimatized before being put into the aquarium. Seaweed used in this study came from Surabaya city. The type of seaweed used in this study is Gracilaria sp. Seaweed will be acclimatized before being put into the experimental tank. During the research, water quality measurements were carried out which included temperature, pH, DO, salinity, ammonia, nitrite, and nitrate every day.

2.4. Statistical Analysis

Data of the levels of heavy metals in seaweed, blood cockles, and water were analyzed by *Analysis of Variance* (ANOVA). If the results of the statistical analysis show that the effect is significantly different or very different, it is followed by Duncan's Multiple Range Test.

3. RESULT

The results of lead (Pb) concentration reduction on seaweed, blood cockles, and water of Biofilter for 7 days can be seen in table and graph 1.

Table 1 The Mean of lead (Pb) concentration reduction on seaweed, blood cockles, dan water of Biofilter.

	Lead (Pb) Concentration in (mg/l)		
Treatments	Seaweed Ratio (%) ± SD	Blood Cockles Ratio ± SD	Water Ratio (%) ± SD
P0	$0.3545^{b} \pm 0.0933$	$0^{c} \pm 0$	$1.2630^{b} \pm 0.1307$
P1	$0.4023^{b} \pm 0.2803$	$1.4715^{a} \pm 0.9474$	$0.3415^{a} \pm 0.2572$
P2	$1.4280^{a} \pm 0.2865$	$0.3290^{b} \pm 0.2364$	$0.0736^{a} \pm 0.1029$
P3	$0.4035^{b} \pm 0.3395$	$0.4180^{b} \pm 0.2895$	$0.2643^{a} \pm 0.1844$
P4	$0^{c} \pm 0$	$0.4235^{b} \pm 0.2032$	$0.1900^{a} \pm 0.1783$

* : P0 (100% *Gracillaria sp.*), P1 (75% *Gracillaria sp.* 25% *Anadara granosa*), P2 (50% *Gracillaria sp.*, 50% *Anadara granosa*), P3 (25% *Gracillaria sp.*, 75% *Anadara granosa*) and P4 (100% *Anadara granosa*). Different superscripts show significant differences (p<0,05).

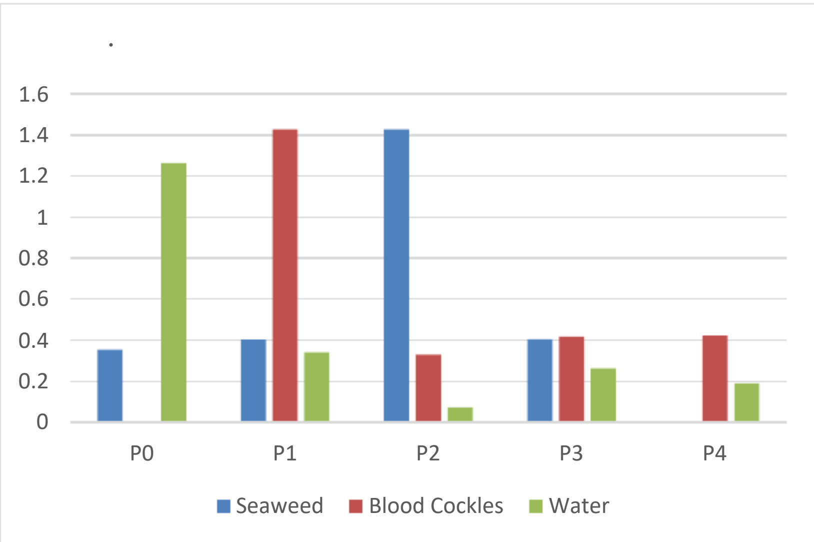

Figure 1. The Mean of lead (Pb) concentration reduction in seaweed, blood cockles, dan water of biofilter.

Data show the mean of lead (Pb) concentration reduction for 7 days in P0, P1, P3, P4 treatments significantly with P2 treatments. Results of ANOVA test shows that the combination of biofilter with seaweed and blood cockles with zeolite showed a difference ($p < 0.01$) on the Pb concentration of Seaweed (*Gracilaria sp.*), so it was continued to Duncan's Multiple Range Test.

Table 1 shows that all treatment was a significant difference and P2 treatment is the best treatment with 50% seaweed and 50% blood cockles. The results of Duncan's Multiple Range Test show that the best of Pb concentration of Seaweed was P2 treatment (mean = 1.42). Pb concentration showed significant differences to all treatment (P0, P1, P2, P3, P4) and P4 treatment was the best treatment with 50% seaweed and 50% blood cockles.

Data of water quality of Nile Tilapia maintenance can be seen in Table 2.

Table 2 Range of water quality in 7 days of Biofilter maintenance

Parameters	Range
Temperature (^{0}C)	28.4-29.9
pH	7.6
DO (mg/l)	1.4-4.0
Salinity (ppt)	20

The water quality parameters observed were temperature, pH, DO and salinity levels in biofilter for 7 days. The results showed that temperatures ranged from 28.4^o-29.9^{o}C, pH (degree of security) ranged from 7-8, DO (dissolved oxygen levels) ranged from 1.4-4.0 mg/l and level of salinity ranged from 15-30 mg/l.

4. DISCUSSIONS

Heavy metal pollution is very dangerous for the environment. Environmental Pollution is any discharge of material or energy into water, land, or air that causes or may cause acute (short-term) or chronic (long-term) detriment to the Earth's ecological balance or that lowers the quality of life (Sastrawijaya, 2009). A biofilter is a wastewater treatment system that is carried out by flowing wastewater into a biological reactor which is filled with a filter to reproduce contaminant decomposing microorganisms contained in wastewater either using aeration or without aeration (Filliazati, 2013).

In this study, the most significant results were *Gracillaria sp.* because of *Gracillaria sp.* has a high ability to absorb heavy metals because the cell walls of *Gracillaria sp.* contain polysaccharides (Yulianto et al., 2006). This is due to the similarity of properties between Pb heavy metals and essential metals in terms of overall chemical properties (Yulianto et al., 2006). Polysaccharides contained in *Gracillaria sp.* is called agar. The agar is formed from a mixture of two polysaccharides, namely agarose and agaropectin (Alamsjah, 2010; Phillips and Williams, 2009).

Accumulation of heavy metals occurs because of the polysaccharides on the cell walls of Gracilaria sp. can bind heavy metal ions and form complex compounds with organic substances contained in the talus (Hurd et al., 2014). In Blood cockles (*Andara granosa*), they are able to act as biofilter because they have filter feeder properties where they consume everything and heavy metals in water are mostly in the form of ions and heavy metals are absorbed from water that passes through the gill membrane or through food. Besides going through gills, heavy metals also enter through the skin (cuticles) and mucous layers then transported by blood and can accumulate in the heart and kidney of blood cockles (Prasetyo, 2009).

Blood cockles have the ability to accumulate heavy metals in the body, so the heavy metal concentration in the body of the blood cockles will increase continuously as long as living in waters containing heavy metals. Even the heavy metal concentration in the body of blood cockles can be higher than that of the environment (Hutagalung, 1994). From the statement above, are in accordance with the results of study where the combination of biofilter which can reduce the Pb heavy metal content in NPK fertilizer waste, which is the most effective combination shows at P2 treatment group with the Pb metal content in the final water 0.0736 ppm with the initial water 1,155 ppm, with proportion of 50:50, *Gracillaria sp.* applied first after that *Anadara granosa* can be applied. This proves that blood cockles and seaweed can absorb and work together well in absorbing Pb heavy metals. Where seaweed absorbs more than 0.908 ppm and shells at 0.329 ppm. This proves that *Gracillaria sp.* has a greater influence (Handhani et al., 2017). *Gracillaria sp.* is a macroalga that has high adaptability to changes in water quality. High adaptability to changes in water quality makes *Gracillaria sp.* able to survive in the stress of heavy metals without disrupting the growth rate.*Gracillaria sp.*

Gracillaria sp. also has a tolerance limit in accumulating heavy metals, if the levels of heavy metals in the talus are too high and for a long time, the growth activity in *Gracillaria sp*. will decline (Yulianto et al., 2006) blood cockles have filter feeder properties that can still absorb heavy metals that do not bind to seaweed. In accordance with the statement, one way to reduce the Pb metal content in waters is to use biofilter, which is a method carried out by utilizing living organisms which aim to reduce pollution in an aquatic environment that contain toxic materials (Yulianto et al., 2006). The ability to accumulate heavy metals make blood cockles can be used as a bio-indicator of heavy metal pollution (Alamsjah et al., 2018; Kadang, 2005).

The aquatic environmental factors that affect the toxicity of heavy metals include temperature, salinity, acidity (pH) and Dissolved Oxygen (DO). These parameters are used to determine the quality of water (Yuan et al., 2017). Where in this study the DO concentration keeps declining over time and heavy metals absorbed by seaweed and blood cockles increased, this is because dissolved oxygen concentration affects the level of toxicity of heavy metals. If the dissolved oxygen (DO) concentration is high, the toxicity of heavy metals decreases, but conversely if the dissolved oxygen (DO) is low then the toxicity of heavy metals increases. From the statement above, it was in accordance with the results of research where high dissolved oxygen (DO) can reduce Pb heavy metal toxicity in NPK fertilizer waste, one of them is in P2 treatment group with water quality parameters at 2.2 mg/l. This shows that a high dissolved oxygen concentration can affect the level of toxicity of Pb heavy metals in water.

5. CONCLUSION

The results of this study that has been done on the combination effectivity of lead (Pb) heavy metal biofilter on NPK fertilizer waste with the best combination in P2 treatments with a proportion of 50% seaweed (*gracillaria sp.*), 50% blood cockles (*anadara granosa*) and Zeolite. The combination of seaweed (*gracillaria sp.*) and blood cockles (*anadara granosa)* as biofilter can be used as an alternative in a wastewater treatment system to reduce lead (Pb) pollution which is very dangerous for the environment.

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