The Effectiveness of Combining Gracilaria Sp. Seaweed Biofilter and Anadara granosa Shell with Zeolite in the Decrease in the Level of Mercury (Hg) Heavy Metal

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Abstract. Biofilter is a wastewater treatment system implemented by flowing waste water into a biological reactor filled with filter media to reproduce contaminant decomposing microorganisms contained in wastewater using aeration or without aeration. This study aims to determine the appropriate combination to reduce the heavy metal content of mercury (Hg). This study used an experimental method with a completely randomized design consisting of five treatments and four replications. The treatment given was the difference in the weight composition of Gracilaria sp. and Anadara granosa for combination. In this study, the number of heavy composition is: treatment P0 (100% Gracilaria sp.), P1 (75% Gracilaria sp. 25% Anadara granosa), P2 (50% Gracilaria sp., 50% Anadara granosa), P3 (25% Gracilaria sp., 75% Anadara granosa) and P4 (100% Anadara granosa). The parameters observed in this study were levels of Mercury heavy metal in seaweed, shellfish and water. Data analysis was carried out using Variant Analysis (ANOVA) and continued with Duncan's Multiple Distance Test. The results show that the use of a combination of Gracilaria sp. and Anadara granosa can absorb Hg heavy metal in water that has been given 1ppm mercury and get the most optimum results in P2 with a composition of 50% Seaweed (Gracilaria sp.), 50% Blood Shellfish (Anadara granosa) and Zeolite.

Keywords: Biofilter Combination, Gracilaria sp, and Anadara granosa.

1. Introduction
Heavy metal pollution is a big threat to the environment. In line with the times, many industrial developments have been found to have liquid waste products in Indonesia. This can enable the increasing concentrations of heavy metals in the waters. One example of heavy metals that continues to increase in concentration is mercury (Hg). Mercury is a heavy metal that has a high level of toxicity (Nirmala et al., 2012).

The use of mercury is very wide with ± 3,000 types in number. Mercury is commonly used in industrial processing of chemicals, the process of making medicines used by humans as well as the basic ingredients of making insecticides for agriculture (Christian et al in Alfian, 2006). All forms of mercury in the form of methyl and in the form of alkyl which enter the human body continuously will cause permanent damage to the brain, liver and kidneys (Roger, et al in Alfian, 2006).

Sea water is usually used by humans to carry out aquaculture activities. But with increasing levels of heavy metals in river water, fish farmers are anxious about their cultivation. Mercury heavy metal (Hg) not only pollutes parts of the body of water but also can pollute parts of the sediment and settle in the body of other aquatic biotas (Nirmala et al., 2012). Based on SNI 7387:2009, the maximum limit...
of Mercury heavy metal (Hg) contamination in water is 0.005 ppm (Badan Standartisasi Nasional, 2004).

Water treatment using filtration is a technology that is economical and easily applied by the community. Adsorption or absorption in general is the process of collecting objects contained in a solution between two surfaces (Sofiyudin, 2015). One example of filtration media that can treat water is zeolite (Nugroho and Setyo, 2013). Zeolite as an ion exchange because of the presence of alkali and alkaline earth metal cations. The addition of biofilter in the filtration process is expected to further optimize the absorption of heavy metals in the waters.

Seaweed has natural phytoextraction, part of the thalus wall of *Glacilaria* sp. will absorb and store organic material which will be produced by the depuration process and will be stored in the thalus cell section (boyajian and carrier, 1997). After storing the waste, the organic material will be degraded through the aid of sunlight photosynthesis and then it will be extracted which results in an energy and cell reflection from the seaweed plant (Burken and Schnoor, 1997).

In addition to using from the type of macroalgae, namely seaweed, it can be used from the bivalves to overcome the accumulation of excess heavy metals in the waters. This ability makes *Bivalvia* a bioindicator of a waters. The process of accumulating heavy metals in the bivalve body is called bioaccumulation or biomagnification. This process is related to the characteristics of these bivalves, namely, sessile (low mobility) or settling on sediments which are dwellings or habitats and are filter feeder biota. One type of shellfish is blood clams (*Anadara granosa*) (Dahuri et al. 1996 in Umar et al. 2001).

Based on the above statements, it is necessary to do research whether the combination of zeolite added with *Gracilaria* sp. seaweed biofilter and *Anadara granosa* shellfish in the filtration process can reduce the accumulation of mercury content (Hg) in waters. So that the waters become suitable for cultivation.

2. METHODOLOGY

2.1. Research Method

This research is an experimental research, which is a way to find a causal relationship between two factors intentionally caused by researchers by eliminating or reducing or eliminating other disturbing factors (Arikunto, 2006).

2.2. Research Time and Place

This research activity was conducted from April 1 to April 20, 2017 at the Faculty of Fisheries and Marine Universitas Airlangga, Surabaya.

2.3. Tools and materials

The tools used during the study were 20 aquariums measuring 40 cm x 30 cm x 30 cm which had been arranged to have 3 holes perforated, aerators, nets, scales, water quality gauges (thermometers, refractometers, pH meters and DO meters).

The material used in the research is virgin shellfish (*Anadara granosa*) originating from waters in the Sedati region of Sidarojo. Dara Shells (*Anadara granosa*) used are 15 grams in size. In each aquarium there is a combination of different levels between shellfish (*Anadara granosa*) and seaweed (*Gracilaria* sp.). The media for maintaining shellfish (*Anadara granosa*) and seaweed (*Gracilaria* sp.) is 10 liters of seawater with 30 ppt salinity that has been treated so that it contains Mercury heavy metal (Hg) of 1 ppm. Zeolite is also used in each treatment, zeolite used is active zeolite.

2.4. Research procedures

The research method used in this study is the experimental method, which is to determine the effect of combination of clams and seaweed (*Gracilaria* sp.) with different percentages of A, B, C, D and E but the same zeolite levels for decreasing metal mercury levels for 7 days of maintenance period. The design of the study used was a Completely Randomized Design (CRD). Completely Randomized
Design used in this study because the maintenance media and place are similar or homogeneous (Sastrosupadi, 2000).

This study used five treatments with four replications, they are:

1. P0 : The combination of biofilter with 100% seaweed (Gracilaria sp.) and zeolite
2. P1 : The combination of biofilter with 75% of seaweed (Gracilaria sp.), clams (Anadara granosa) 25% and zeolite
3. P2 : The combination of biofilter with the provision of seaweed (Gracilaria sp.) 50%, shellfish (Anadara granosa) 50% and zeolite
4. P3 : The combination of biofilter with 25% seaweed (Gracilaria sp.), virgin shellfish (Anadara granosa) 75% and zeolite
5. P4 : The combination of biofilter with clams (Anadara granosa) 100% and zeolite

2.5. Tools and Materials Preparation

The initial preparation of the aquarium to be used is washed with clean water and then dried. Clean-washed equipment is soaked with a chlorine solution of 150 mg/l for 12-24 hours and to remove the odor of chlorine and dirt, washing the aquarium using detergent with the intention of removing parasites in the aquarium (Prakosa 2013).

The aquarium is then filled with 10 liters of sea water with a salinity of 30 ppt and has a heavy metal content of mercury (Hg) of 1 ppm. Then installed, water pumps, bulkhead nets, virgin shells (Anadara granosa), seaweed (Gracilaria sp.) and zeolite in each aquarium. Hg was used at 1 ppm because of Anadara granosa, and Gracilaria sp. the exposure limit for mercury is only 1 ppm (SNI 7387:2009)

Zeolites used in this study are 50gr for each treatment. According to Ashari (2016) on the administration of 50gr zeolite to 10 liters of water containing 1 ppm HG, zeolite has a very significant effect on decreasing the concentration of heavy metals.

The clams (Anadara granosa) to be used are obtained from fishermen in the waters of Sedati Sidoarjo, shellfish (Anadara granosa) brought alive. The clams (Anadara granosa) which will be used as acclimatized filters are then used as experimental materials. Dara Shells (Anadara granosa) used 420 g for 100% filters, 315 g for 75% filters, 210 g for 50% filters and 105 g for 25% filters.

Seaweed used is a type of Gracilaria sp., this species is obtained from the waters of Troncol Sidoarjo, seaweed (Gracilaria sp.) Which is brought alive. Seaweed (Gracilaria sp.) which will be used as a filter was acclimatized beforehand then used as experimental material. Seaweed (Gracilaria sp.) used as much as 340g for filters 100%, 255g for filters 75%, 170g for filters 50%, 85g for filters 25%.

This study uses five aquariums, each aquarium consisting of a combination of shellfish biofilter (Anadara granosa), seaweed (Gracilaria sp.) and zeolite. Then the treatment is done for 7 days and checking the water quality, such as PH, Salinity, Temperature, DO, nitrite, nitrate, carried out every day.

2.6. Maintenance of a combination of virgin shellfish biofilter (Anadara granosa), seaweed (Gracilaria sp.), and zeolite

The shellfish (Anadara granosa) used for the study were virgin shellfish (Anadara granosa) which originated from Sedati waters weighing 7-8 grams/ head. The mussels that will be kept are cleaned first then acclimatized before being put into the aquarium. Seaweed (Gracilaria sp.) used in this study came from Ayu's Medokan area, Rungkut, Surabaya. The type of seaweed (Gracilaria sp.) used is Gracilaria sp. Seaweed (Gracilaria sp.) which will be maintained will be acclimatized before being put into the experimental aquarium.

During the research, water quality measurements were carried out which included temperature, pH, DO, salinity, ammonia, nitrite and nitrate every day.

2.7. Data analysis
This study uses a completely randomized design (CRD) method to determine the effect of the treatment given. In this design there is only one source of diversity, namely treatment in addition to random influences, so the results of differences between treatments are only caused by the effects of treatment and random effects only. (Kusmaningrum, 2008).

This study used five treatments and four replications. The effect of the combination of shellfish biofilter, seaweed with zeolite showed significant results, the calculation will be continued with Duncan’s Multiple Range Test (Kusriningrum, 2012).

3. Results and discussion
3.1. Results
3.1.1. Bioaccumulation of Seaweed (Gracilaria sp.) Against Mercury (Hg)
Gracilaria sp. seaweed used for this study was obtained from Medokan Ayu area, Rungkut, Surabaya. In a fresh state with the dark green complexion in characteristics.

The results of the content test on seaweed before being used for biofilter combination studies of 0.095 ppm, testing the content of Hg heavy metal on Gracilaria sp. Seaweed aims to compare the content of Hg heavy metal in Gracilaria sp. Seaweed after biofilter. The following data on average and SD results from the final results of Gracilaria sp. can be seen in Table 1.

| Treatment | Average ± SD    |
|-----------|----------------|
| P0        | 0.2175\(^b\) ± 0.0602 |
| P1        | 0.3050\(^bc\) ± 0.0580 |
| P2        | 0.3600\(^c\) ± 0.0594 |
| P3        | 0.1575\(^a\) ± 0.0512 |
| P4        | 0 ± 0           |

Table 1. Average absorption of Gracilaria sp. in Hg heavy metal.

Description: Treatment of P0 Combination of Gracilaria sp. 100% with Zeolite, P1 Combination of Gracilaria sp. 75% Anadara granosa with Zeolite, P2 Combination of Gracilaria sp. 50% Anadara granosa 50% with Zeolite, P3 Combination of Gracilaria sp. 25% Anadara granosa 75% with Zeolite, P4 100% Anadara granosa with Zeolite.

Graph of Hg heavy metal content in Gracilaria sp. before and after biofilter can be seen in Figure 1.

Figure 1. Graph of Hg heavy metal content in Gracilaria sp. before and after the study.

Description: P0 = 100% Seaweed
P1 = 75% Seaweed
P2 = 50% Seaweed
P3 = 25% Seaweed

The graph above shows that *Gracilaria* sp. seaweed is able to absorb Hg heavy metal so that the content of Hg heavy metal in *Gracilaria* sp. seaweed shows an increase. From the graph above, it can be seen that the Hg content in *Gracilaria* sp. seaweed in P2 treatment, which is 0.360 ppm, is able to absorb Hg heavy metals with optimum compared to P3 treatment which is 0.158 ppm, P1 is 0.305 ppm, and P0 is 0.218 ppm. The ability to absorb with optimum treatment P2 was obtained from a combination with *Anadara granosa* shellfish 50%: 50%.

### 3.1.2. Bioaccumulation of *Anadara granosa* shells against Hg heavy metal

The blood clams of *Anadara granosa* used for this research were obtained from the Sedati Subdistrict, Sidoarjo District, East Java Province. With fresh state and the characteristics of it is having 2 shell valves, red and soft. Length, weight and width measurements were taken to determine the morphometrics in the blood shells and organoleptic tests for the results of shellfish that had accumulated Hg heavy metal were measured. The average results and SD *Anadara granosa* can be seen in Table 2.

#### Table 2. Average absorption of *Anadara granosa* in Hg heavy metal.

| Treatment | Hg content in *Anadara granosa* (mg/l) | Average ± SD |
|-----------|--------------------------------------|--------------|
| P0        | 0 ± 0                                |              |
| P1        | 0.2975 ± 0.03096                     |              |
| P2        | 0.4150 ± 0.04933                     |              |
| P3        | 0.3150 ± 0.02646                     |              |
| P4        | 0.3625 ± 0.01708                     |              |

Description: Treatment of P0 Combination of *Gracilaria* sp. 100% with Zeolite, P1 Combination of *Gracilaria* sp. 75% *Anadara granosa* with Zeolith, P2 Combination of *Gracilaria* sp. 50% *Anadara granosa* 50% with Zeolith, P3 Combination of *Gracilaria* sp. 25% *Anadara granosa* 75% with Zeolith, P4 100% *Anadara granosa* with Zeolith.

Based from the results of the study, the collected data with the best absorption was found in P2 treatment with *Anadara granosa* 50% shellfish biofilter and *Gracilaria* sp 50%.

Graph of Hg heavy metal content in *Anadara granosa* virgin shells can be seen in Figure 2.
Figure 2. Graph of Hg heavy metal content in *Anadara granosa* before and after the study.

Description:  
P1 = 25% Shells  
P2 = 50% Shells  
P3 = 75% Shells  
P4 = 100% Shells

From the results of the study, the above graph shows that *Anadara granosa* blood clams can absorb Hg heavy metals so that the heavy metal content of Hg in water decreases due to the ability of *Anadara granosa* blood clams to absorb heavy metals and the heavy metal content of shellfish to increase. From the graph above, it can be seen that the clam content in P1 is 0.2975 ppm, lower than the blood clam content of *Anadara granosa* P4, which is 0.3625 ppm and P3 0.315 ppm because the shellfish found in P3 and P4 treatments are more than P1. The optimum absorption occurred in P2 treatment which is 0.415 ppm.

3.1.3. Decrease in Hg heavy metal in water  
From the results of the study the effectiveness of the combination of biofilter can be seen from the decrease in heavy metals in water which has been inoculated with Hg heavy metal used in the study. Test results for the content of Hg heavy metal in water before a biofilter combination of 1,155 ppm. The average and elementary results of the decrease in the content of Hg heavy metal in water can be seen in Table 3.

| Treatment | Average ± SD | Content of Hg Heavy Metal on Water (mg/l) |
|-----------|--------------|----------------------------------------|
| P0        | 0.029bc ± 0.0086 |                                      |
| P1        | 0.0250bc ± 0.00560 |                                    |
| P2        | 0.0175bc ± 0.00411 |                                    |
| P3        | 0.0215bc ± 0.00404 |                                    |
| P4        | 0.0258bc ± 0.00457 |                                    |

Description: Treatment of P0 Combination of *Gracilaria sp.* 100% with Zeolite, P1 Combination of *Gracilaria sp.* 75% *Anadara granosa* with Zeolith, P2 Combination of *Gracilaria sp.* 50% *Anadara granosa* 50% with Zeolith, P3 Combination of *Gracilaria sp.* 25% *Anadara granosa* 75% with Zeolith, P4 100% *Anadara granosa* with Zeolith.
From the data above, the decrease in Hg heavy metal in each treatment showed that seashells and seaweed could absorb heavy metals with optimum treatment for each.

Graph of decreasing Hg heavy metal content in water can be seen in Figure 3. And complete data of Hg heavy metal before and after can be seen in the attachment. The best reduction in Hg heavy metal in P2 treatment containing Hg heavy metal was at least 0.0175 ppm compared to the other treatments.

![Graph of decrease in Hg heavy metal content after and before on water.](image)

**Figure 3.** Graph of decrease in Hg heavy metal content after and before on water.

**Description:**
- **P0 =** 100% Seaweed (*Gracilaria* sp.) and Zeolite
- **P1 =** 75% Seaweed (*Gracilaria* sp.), 25% Blood Shellfish (*Anadara granosa*) and Zeolite
- **P2 =** 50% Seaweed (*Gracilaria* sp.), 50% Blood Clams (*Anadara granosa*) and Zeolite
- **P3 =** 25% Seaweed (*Gracilaria* sp.), 75% Blood Clams (*Anadara granosa*) and Zeolite
- **P4 =** 100% Blood Shells (*Anadara granosa*)

The graph above shows that P2 treatment with the content of 0.0175 ppm is better in the treatment of P2 combination of 50% Seaweed and 50% green mussel with absorption of 0.285 ppm, with 100% Seaweed content, after that P4 is 0.258 ppm, P3 is 0.215 and the last is P1 0.025 ppm.

### 3.2. Discussion

Heavy metal pollution is very dangerous for the environment. Environmental pollution occurs because of the entry or inclusion of living things, substances, energy and/or other components into the environment (Sastrawijaya, 2000). According to Effendi (2003) heavy metals have non-degradable properties. In addition, heavy metals will accumulate in the environment such as water and sediment columns and be absorbed into marine biota.

This research was conducted with one of the marine biota, namely *Gracilaria* sp. seaweed because *Gracilaria* sp. has the ability to absorb high heavy metals since its cell walls contain polysaccharides (Yulianto et al., 2006). As well as added shells as a filter feeder that will accumulate heavy metals in the body of the shells (Lestari, 2002). With a biofilter system, a wastewater treatment system is carried out by flowing waste water into a biological reactor which is filled with filter media to reproduce contaminant decomposing microorganisms contained in wastewater using aeration or without aeration. (Filliazati et al., 2013).

From the results of the study, optimum absorption of Hg heavy metal was found in P2 treatment with a composition of 50% seaweed and 50% green mussel with absorption of 0.4150 ppm and 0.2850. The combination with a percentage of 50%: 50% seaweed and shellfish can absorb perfectly in water inoculated with Hg heavy metal according to Yulianto’s statement (2006) that in *Gracilaria* sp. it is suspected that ion exchange also occurs, where ions enter the cortex in which there are cells with various functions. But not all of these ions are associated with cells in the *Gracilaria* sp seaweed. It can be seen from the metal content left in the water.
Anadara granosa shells that have the ability as a filter feeder are also able to accumulate heavy metals in their organs. Shellfish have bioaccumulative properties of heavy metals. Heavy metals in the water will enter into the food chain cycle or flocculate in the "metal-humate" compound, so that it accumulates and undergoes a biological level increase (biomagnification) in the animal's body and substrate. At certain levels of metal contained in the body of an animal can interfere with body organs or become toxic and can be fatal to the organism (Tetelepta, 1990).

In treatment P4, which only contained 100% of the clam organism Anadara granosa, the absorption of heavy metal 0.3625 ppm was assumed that the mussels could not accumulate Hg heavy metal in the body organs perfectly. In treatment P0, which only contained 100% of seaweed Gracilaria sp, there was an absorption of 0.2175 ppm from the treatment, presumably because Hg heavy metal excreted through urine so that it accumulated in water according to Hutagaluang (1998) who stated that organisms can absorb heavy metals and can reissue heavy metals through the body surface, stomach contents, gills or urine.

In treatment P1 which uses seaweed (Gracilaria sp) 75%, shellfish (Anadara granosa) 25%, seaweed absorption is 0.3050 ppm and shellfish is 0.2975 ppm. Gracilaria granosa absorbs more than shellfish because the amount of seaweed is more than that of shellfish and vice versa in P3 treatment using seaweed (Gracilaria sp.) 25%, shellfish (Anadara granosa) 75% occurring seaweed absorption of 0.2850 ppm and shells of 0.3150 ppm but each organism has a tolerance limit to heavy metals.

In accordance with the statement of Yulianto et al. (2006) Gracilaria 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 Gracilaria sp. and Anadara granosa shells is slightly different which has filter feeder properties that can accumulate heavy metals but also can come out through urine.

The accumulation of Hg heavy metals was seen organoleptically in the Anadara granosa and Gracilaria sp shells according to Phillips (1980). This is indicated by several appearances, such as the thallus which becomes non-elastic (easily broken) and yellowish tip of the thallus. This is thought to be the occurrence of plant physiological disorders due to the inability of plants to tolerate the high concentration of test media by metal Hg that the entry of heavy metal elements into the body of seaweed results in compounds between metals and proteins and polysaccharides which then penetrate the cell wall and enter the cytoplasm.

The Anadara granosa shells also appear to indicate that the accumulation of Hg heavy metals in Anadara granosa's organs occurs in a deterioration of quality in a short period of time with foul-smelling clams with slightly open shells (Porsepandi, 1998).

The addition of zeolite to each treatment did not cause a significant difference in the content of Hg heavy metal because zeolite became a medium attached to microorganisms, to form a biological layer (biofilm) which functions to decompose organic matter (Budijono et al., 2010).

Aquatic environmental factors that affect the toxicity of heavy metals include temperature, salinity, acidity (pH) and dissolved oxygen or Dissolved Oxygen (DO) and these parameters are used to determine the quality of a water (Darmono, 2006).

Where in this study the longer and lower the heavy metal absorbed by seaweed with shellfish increases because the value of dissolved oxygen content affects the level of toxicity of heavy metals. If the dissolved oxygen (DO) content is high, the toxicity of heavy metals decreases, but conversely if the dissolved (DO) oxygen is low then the toxicity of heavy metals increases.

In this study, statistical tests were tested with Completely Randomized Design (CRD) tests, which were experimental, namely this design is commonly used for experiments that have uniform or homogeneous media or experimental environments. (Mattjik & Sumertajaya, 2000).

4. Conclusions and suggestions
4.1. Conclusion
Based on the results of the research, the use of a combination of Gracilaria sp. and seashell Anadara granosa seaweed biofilter can reduce levels of mercury (Hg) with the combination of Gracilaria sp. Anadara granosa with Zeolite as follows:
The use of the combination of *Gracilaria* sp. and *Anadara granosa* can absorb *Mercury* (Hg) *heavy metal* with the most optimum combination in P2 with a composition of 50% Seaweed (*Gracilaria* sp.), 50% Blood Shellfish (*Anadara granosa*) and Zeolite.

4.2. Suggestion

It is expected that in the next study, a detailed study of the ability to absorb the shells of *Anadara granosa* and *Gracilaria* sp. against mercury (Hg) heavy metal including internal organs is conducted.

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