The effect of water immersion on decreasing copper (Cu) and granulocyte levels in *Crassostrea cucullata*

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Abstract. Oyster habitat in a body of water often contain excessive metal content. This research aimed to decrease of Cu level concentration and understand the change of granulocyte in oyster body after being treated for sea water for 12 hours. This research used experimental method with Completely Randomized Design consisting 4 treatments and 3 replications. Cu concentration was measured by using AAS method and the amount of hemocyte (granulocyte) was calculated by using haemocytometer. Cu concentration in oyster before treatment was 0.374 ppm. Immersion with the water discharge of 0.05 m/s for 12 hours was able to decrease Cu 46% (0.158 ppm of Cu residue) with the lowest granulocyte (31.92%).

1. Introduction

1.1. Background

Coastal waters contain copper (Cu) of 0.063 ppm, and the level measured in *Crassostrea cucullata* is 0.374 ppm. According to the Regulation of the Minister of Environment of the Republic of Indonesia No. 51 2004 stated that, the Cu level in marine biota should be 0.008 ppm. Thus, the Cu concentration found in *Crassostrea cucullata* is under the standard quality. The amount of Cu in oysters can be reduced by immersing water. The immersion of *Crassostrea cucullata* in seawater sterilized with chlorinfor 3 days can significantly decrease the total number of hemocytes, thus the oyster’s condition improves after the first 12 hours of immersion [1]. If the oyster’s body contains a high level of heavy metal, the production of hemocytes will increase [2]. Oyster hemocytes consist of hyalinocytes, that recognize foreign materials, and granulocytes, that perform phagocytosis.

Copper or Cu in oysters is required for the formation of haemocyanin (oyster blood), and the permissible level is only 0.008 ppm. The absorption of Cu into the blood can occur in acidic conditions in the stomach [3]. An environment with toxic conditions will trigger the body of an oyster to adjust its metabolism by performing excretion or detoxification. The natural detoxification process begins with the binding of metal ions on the cell surface, since positive ions are bound to the reactive side to the negative charge of the extracellular polymer. The next stage is the transport of metal ions to the cytoplasm, and then the metal ions are accumulated by metallothioneins or MT proteins [4].

Various animal physiological devices such as rates of excretion and metal detoxification are typical of each species [5]. Not all organisms are able to excrete metals. Therefore, the rate of detoxification in animals becomes the only balancer of the uptake rates to prevent metal toxicity. When metal first enters the body, metal regulation immediately begins, and the metal ions are circulated throughout the body by the hemolymph fluid [6]. Toxic metals are excreted, while un-excreted metals will
accumulate in the body. The accumulated metals will be regulated through metabolism or released through detoxification or excretion. All non-essential metals (including heavy metals) will be detoxified by the animal body by being strongly bonded to a particular site that is difficult to be removed.

By immersing clean water can dissolve the protein in the oyster body. The use of destilled water as a solvent can reduce protein levels because aqua is able to dissolve and modify the properties of biomolecules such as nucleic acids, proteins and carbohydrates through the formation of hydrogen bonds with polar functional groups [7]. According to Suaniti [8], the heavy metal concentrations are reduced byreleasing metal-protein complex bonds, so that the metal ions are out of the oyster stomach. Metal ions are naturally present in the animal body, and almost all of them bind to proteins.

2. Methodology
This research was conducted by using experimental method with Completely Random Design and consisted of four treatments of water flowing (3-hour, 6-hour, 9-hour, 12-hour flow) and 3 replications. Oysters were sterilized by water sterilized with chlorine at a rate of 0.05 m/sec. Phytoplankton observations were conducted in the original habitat of oysters to determine the amount of feed administered in the treatment media, that was 9.070 cells/L. The height of the tidal water was also measured to determine the average water content of the treatment media, which was 30 cm in average. In addition, the oysters and water samples were taken to observe the levels of granulocyte and copper (Cu) prior to treatments.

The pail for treatment was washed and chlorinated first, and it was given certain hole to adjust the water flow at the outlet. Furthermore, water filling in each treatment pailwas done at a height of 30 cm in accordance with the condition of the oysters’ habitat. Each ± 20 liter basin was given an aerator and filled with four oysters. Water flowing was done for up to 12 hours based on the tides. Oyster sampling was done every 3 hours for measurement of granulocyte and Cu as well as water quality (temperature, pH, dissolved oxygen, and salinity).

3. Results and Discussion
3.1. Analysis of Cu and granulocyte in Crassostrea cucullata
The levels of Cu and granulocyte decreased after the oysters were immersed by water for up to 12 hours. The granulocyte level decreased by 31.92 %, and the Cu level decreased to 46 % (figure 1). Treatments of 3-hour, 6-hour, 9-hour and 12-hour had significantly decreased the granulocyte and Cu levels in oysters. The best treatment was 12-hour because the granulocyte level decreased by 31 %, and the remaining Cu level was 46 %.

![Figure 1. The Average of Cu and Granulocyte in Crassostrea cucullata after 3 hours, 6 hours, 9 hours and 12 hours Immersion.](image-url)
3.2. Water quality parameters

Water quality parameters such as temperature, pH, dissolved oxygen, and salinity at the sampling location and immersion treatment location were in the normal range (table 1). However, there was a difference of 5.8 °C between the sampling location and the treatment location.

Table 1. Water quality analysis result.

| Water Quality | Observation Result | Standard |
|---------------|--------------------|----------|
|               | Oyster Habitat     | Laboratory |         |
| Temperature (°C) | 30.5               | 24.7-26.3 | 28.32 [12] |
| pH            | 8                  | 8.3-8.5   | 7.8-8.6 [13] |
| DO (mg/L)     | 2.35               | 7.62-8.43 | >1 [14] |
| Salinity (ppt)| 33                 | 32.5-35   | 27-33 [12] |

Water quality becomes one of the factors that supports oyster life. When the research was conducted, the water quality parameters were in the range appropriate for oyster life. The difference between the temperature of the sampling location and that of the treatment location was 5.8 °C, which was still in the range of the appropriate temperatures for oyster life [12]. Bivalves are able to live in waters at pH 7.8–8.6 [13]. Oysters are still able to survive for 5 days in waters containing >1 mg/L of dissolved oxygen [14]. The optimal salinity range for bivalves in the waters is 27-33 ppt [12].

The average concentration of Cu in the oysters was 0.29297 ppm, and the number of oyster granulocytes (control) was 50.88 %. The levels of Cu and granulocyte decreased after the oysters were for 12 hours. The granulocyte level decreased 31.92 %, and the Cu level decreased 46 %. If the percentage of granulocytes is 31 %, it means the oyster is in good health or the environment has not been contaminated [9]. The granulocyte levels of bivalves in the uncontaminated waters ranged from 34 %, and the granulocyte levels of bivalves in polluted waters ranged from 52 %. Based on this research, the seosters are safe to consume.

Heavy metals in animal body are absorbed by the blood, bound by blood proteins, then distributed throughout the body tissues [10]. Heavy metals can enter the oyster body in two ways: passive uptake (passive transport) and active uptake (active transport) because heavy metals and essential metals have similar physical-chemical properties [11].

The flowing using seawater over Crassostrea cucullata decreased the Cu and granulocyte levels. The 12-hour flowing was effective to reduce the amount of Cu in oysters to 46 %, and the number of granulocytes by 31 %.

4. References

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