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Phytoremidiation of Pb and Cd using indigenous *Eucheuma cottonii* Madura Island, Indonesia

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ABSTRACT

Water pollution is the event of the entry of substances that are toxic in nature which later can reduce the quality of water that is in a waters. The presence of too many heavy metals Pb and Cd in the waters can cause threatened ecosystems in a waters. Heavy metals of this type enter a lot of water due to sea transportation activities such as diesel oil, industry and coastal community activities. Many studies suggest that *Eucheuma cottonii* seaweed species is effective in absorbing heavy metals Pb and Cd, therefore further research is needed regarding the effectiveness of *Eucheuma cottonii* seaweed species in absorbing differences in Pb and Cd heavy metals. The purpose of this study was to determine the ability of phytoremediation of Pb and Cd using seaweed species *Eucheuma cottonii.* The method used in this study is the experimental method using a Completely Randomized Design (CRD). The procedure of this study included sample preparation, treatment and observation of water quality (temperature, salinity, pH and DO) for 10 days, and testing of heavy metal content in water and seaweed at the beginning and end of the study. The t-test results of environmental parameters on the degradation of Pb and Cd heavy metals with a confidence interval of 95% indicate that Temperature and DO do not have a significant difference but vice versa in the pH and Salinity data.

Key words : *Seaweed, Eucheuma cottonii, Phytoremediation, and heavy metals.*

Introduction

Water pollution occurs because of human actions that can arise from various kinds of human activities, whether intentional or not.

Heavy metals with high toxicity is lead (Pb) and Cadmium (Cd). In everyday life cadmium is used as a metal coating material or electroplating, stabilizer, plastic coloring and industrial batteries. In the aquatic environment, cadmium metal will undergo biotransformation and bioaccumulation in living organisms. The metal content of these organisms will increase (biomagnification) and the highest biota in the food chain will experience the greatest accumulation.

Lead (Pb) and Cadmium (Cd) is one of the non-essential heavy metals which is present in dangerous waters and is toxic to organisms because of its toxic nature. This toxin is cumulative, meaning that the toxicity will arise if it accumulates in large enough quantities in the body of living things. Pb is found in waters due to contact between water and sediment or lead polluted air, or due to waters contaminated by industrial waste.

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Very large lead in the waters can cause behavioral and physiological disturbances in aquatic organisms. The condition of very chronic lead exposure can even cause threats to the digestive organs and the heart (Daud *et al.*, 2015). Heavy metal pollution can be handled by carrying out bioremediation techniques. The types of plants that can carry out remediation are called hyperaccumulator plants (Oves *et al.*, 2012). Hyperaccumulator plants are plants that can absorb heavy metals with a minimum concentration of 1000 g/g of plant biomass. Almost all plant species experience a significant reduction in the weight of biomass if concentrated metals in the body tissues (Reeves, 1992).

Algae can be grouped into 2 groups based on their size. Macroalgae or commonly known as seaweed and microalgae, single cell organisms with sizes varying from a few micrometers (μm) to several hundred micrometers (Milledge *et al.*, 2014). Seaweed has a function both directly and indirectly. Directly or ecologically known seaweed provides food for fish and invertebrates, especially young thallus (Mann, 1982).

Eucheuma cottonii seaweed is widely distributed in the world's marine waters. The use of this type of seaweed is often cultivated for medicinal substances or used in absorbing heavy metals (Abirami and Kowsalya, 2012). Heavy metal wastes such as Pb and Cd can be degraded using seaweed extract. *Eucheuma cottonii* is one of the seaweed species which is quite effective in degrading heavy metals. The purpose of this study was to determine the ability of *Eucheuma cottonii* seaweed species to absorb heavy metals Pb and Cd.

Methods

Sample Preparation

The test material to be used in this study is seaweed species *Eucheuma cottonii* originating from cultivation in Sumenep Regency, Madura. *Eucheuma cottonii* used as much as 15 grams of wet weight which will be placed on each research media jar. The test container for this study was planting media in the form of clear plastic jars with a volume of 10 liters of 16 pieces and each container filled with sea water as much as 5 liters of sample.

Water Quality Observation

The water quality parameters observed in this study included temperature, salinity, pH and dissolved oxygen (DO) for 10 days. This 10 day period is adjusted to the age cycle of seaweed, which is for 30-35 days, where in this study seaweed used was ± 25 days old. If the age of seaweed exceeds 30-35 days, generally seaweed is easily attacked by disease. Temperature measurement using a digital thermometer, salinity using a refractometer, pH using a pH meter, and DO using DO meters. Measurements were made by repetition 3 times with an interval of ± 5 minutes then the average value was taken to get accurate data.

Absorption Percentage of Heavy Metal Pb and Cd

The percentage of *Eucheuma cottonii* and *Sargassum cristaefolium* seaweed in absorbing heavy metals Pb and Cd can be done by comparing the initial heavy metal content of the treatment and after 10 days of treatment on water and seaweed. The percentage value of heavy metal absorption can be used as a comparison of which species are more effective in absorbing heavy metals, as seen from which seaweed species have a greater percentage value. Percentage of absorption of heavy metals Pb and Cd by seaweed *Eucheuma cottonii* measured by following formula:

Notes:
Co = Initial Heavy Metal Concentration (mg/L)
Ce = Last Heavy Metal Concentration (mg/L)

Bio-Concentration Factor (BCF)

Bio-Concentration Factor (BCF) is the result of analysis that is usually used to identify indicators of an organism in absorbing organic and inorganic materials in the environment both land and in water. Bioconcentration factors (BCFs) are parameters that are useful for evaluating the potential of algae in accumulating metals. Calculation of the value of these BCFs is based on dry weight (Lamai *et al.*, 2005). The formula for calculating BCF values is as follows.

The category value of the Bio-Concentration Factor is divided into 3 categories including:

Accumulator	: BCF > 1
Indicator	: BCF = 1
Excluder	: BCF <1

Statistical Test Data Analysis

The statistical analysis methods used were variance (ANOVA: Analysis of Variance) and BNT (Smallest Significant Difference). BNT Analysis (Smallest Sig-

nificant Difference) is used if the results of the variance analysis are obtained F count> F table 5%. Then determine which varieties are more potential by looking at the comparison value of BNT (Smallest Significant Difference). The formulas from BNT are as follows:

Notes :

t = t Table = 5%; N = amount of data; u = treatment; R = test; KT error = Middle Quadrant Error

Results and Discussion

Data on Environmental Parameters (Temperature, pH and DO)

Measurement of environmental parameters for the analysis of Pb and Cd concentrations used did not show a significant difference in value. The largest standard deviation value is owned by the salinity parameter, indicating that the diversity of measured temperature data is relatively high compared to the other parameters.

The results of the t test using the 95% confidence interval indicate that the temperature and DO data on the two heavy metals did not show significant or significant differences. The pH and salinity data on Cd and Pb heavy metals after being tested using the t-test showed a significant difference

Percentage of Heavy Metal Content in Water and Seaweed

The percentage of reduced heavy metal Cd in water is greater than the percentage of heavy metal Pb. This shows that *Eucheuma cottonii* seaweed absorbs Cd more heavy metals than Pb. The percentage difference in absorption of heavy metals Pb and Cd is 22.17%. The difference in the reduction in the percentage of heavy metals Pb and Cd in water is 7.67%.

Based on this, it shows that *Eucheuma cottonii* seaweed has the ability to absorb heavy metals in water. According to Romera *et al* (2006) seaweed has biosorption capabilities of heavy metals in water, but each seaweed species has a different biosorption ability.

Table 1. Data on environmental parameters at the time of observation of heavy metal sealing

Environmental parameters	Pb	Cd
Temperature (^{0}C)	25.64 ± 0.077	25.56 ± 0.175
pH	7.62 ± 0.034	7.57 ± 0.036
DO (mg/L)	5.70 ± 0.015	5.67 ± 0.004
Salinity (ppt)	30.2 ± 0.289	31.47 ± 0.300

* ± : Standard deviation

Effect of Heavy Metals on the Conditions of Seaweed Morphology

The morphological conditions of *Eucheuma cottonii* were given 1 ppm addition of Pb with a exposure time of 10 days in salinity 30 ppt sea water medium still looked normal and healthy at day 0 observation (Figure 1A). Figure 1B shows the thallus condition at the 10th day observation. The thallus of *Eucheuma cottonii* on the 10th day had changed shape and color. Figure 1C is the morphology of *Eucheuma cottonii* seaweed after being exposed to Cd heavy metals (end of observation) and the morphological changes are evident after being exposed to heavy metals 1 mg/L. However, *Eucheuma cottonii* seaweed still survived even though it was exposed to Cd heavy metal by 1 mg / L during the study. Some symptoms will arise due to excess concentrations of heavy metals in the water. One of them will result in a reduction and inhibition of the process of absorption of nutrients by macroalgae, so that the growth process becomes inhibited (Chino, 1981).

As a result of heavy metals in the waters can cause damage to marine biota if the marine biota continuously accumulates these heavy metals, especially in seaweed if the metal content of cadmium (Cd) crosses the threshold it will inhibit seaweed growth and eventually seaweed can damaged (Teheni *et al.*, 2016).

Results of Bio-Concentration Factor (BCF) Value

The ability of plants to tolerate and accumulate heavy metals using bioconcentration factors (BCF),

Table 2. Average results of measurements of heavy metals in water

HM	Concentration t0 (ppm)	Concentration t1 (ppm)	t0 – t1 (ppm)	Loss (%)
Pb	1.1598	0.625 ± 0.006	0.5350	46.13
Cd	1.0256	0.476 ± 0.010	0.5520	53.8

* ± : Standard deviation

Table 3. The average results of heavy metal measurements on seaweed

HM	Concentration t0 (ppm)	concentration t1 (ppm	t0 – t1 (ppm)	Absorbance (%)
Pb	1.1738	0.3045 ± 0.005	0.1307	75.23
Cd	0.0679	0.1341 ± 0.010	0.0662	97.4

*) ± : Standard deviation
Notes:
t0 : Initial concentration of heavy metal
t1 : Last concentration of heavy metals

Table 4. Results of Calculation of BCF Seaweed Value

HM	Average Metal concentration on Seaweed (mg/L)	Average Metal concentration on Water (mg/L)	BCF
Pb	0.3045 ± 0.00454	0.625 ± 0.00596	0.49
Cd	0.1341 ± 0.10	0.4736 ± 0.01	0.28

*) ± : Standard deviation

can be used to determine the status of these plants. The results of BCF calculations (Table 4) are the values obtained from the comparison between the concentration of heavy metal *Eucheuma cottonii* and the concentration of heavy metals in water. Viewed from the BCF category, *Eucheuma cottonii* can be categorized as an excluder plant because it has a BCF value of <1. Plants that are categorized as metal excluder species are plants that absorb heavy metals, while maintaining cell damage by preventing the entry of excess heavy metals from the environment into his body.

Based on the two BCF values above, it can be concluded that this species of *Eucheuma cottonii* can prevent the entry of excess heavy metals Pb and Cd from their environment through adjustments that occur in their tissues.

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