Effectiveness and efficiency of the red seaweed Gracilaria verrucosa as biofilter in Pb absorption in seawater

by Supriyantini Et Al.

Effectiveness and efficiency of the red seaweed *Gracilaria verrucosa* as biofilter in Pb absorption in seawater

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Abstract. Waste, containing heavy metals from industries and other anthropogenic activities are mostly disposed into waters without any treatments, resulting in an increase in heavy metal concentrations. Algae, such as *Gracilaria verrucosa,* are known to have to ability to absorb heavy metal and function as biofilter. This study investigates the effectiveness and efficiency of *G. verrucosa* to absorb heavy metal at various concentrations and at a given exposure period. The experiment used a completely randomized design with 2 factors in 3 replications. First factor was Pb concentrations, namely: 0.0, 0.06, 0.6, 6.0 ppm, whereas the other factor was different time of exposure, namely: 7, 14, 21, 28 days. Data were analyzed statistically with Anova followed by Tukey Test. Results showed that concentration and incubation period influenced algal absorption of Pb. As much as 75.28% of Pb was efficiently absorbed by the red algae during 28 days exposure time with Pb added media by bioabsorption capacity 0.084 $mg.g^{-1}$. This result suggested that red alga *G. verrucosa* can be applied as biofilter for Pb in seawater.
Key Words: heavy metal Pb, macroalgae, Efficiency, absorption capacity.

Introduction. Sources of marine pollution can be derived from industry, agriculture, and domestic wastes. Industrial waste contains more pollutants such as organic substance, inorganic salts, heavy metals, oils etc. (Palar 2008). One of the industrial pollutants contributes more to waters alteration, these are the heavy metals (Goyal and Chauhan 2015), that has been used as either the main or additional material, as well as catalisator agent. These waste disposals into the waters without any standardized waste treatment process will increase concentration of heavy metals in waters. These pollutants will be accumulated in sediment and organisms (Santoro et al 2009).

One of the heavy metals which is toxic to living organism is the lead (Pb) (Jaishankar et al 2014). The presence in high concentrations of Pb in waters can kill aquatic organisms. Concentration of 188 ppm was reported to be lethal for fish and for crustaceans at 2.75-49 ppm after 245 hours period of incubation (Palar 2008). According to the Decree of the Minister of Environment of Indonesia, Number 51 of 2004, the criteria of seawater quality standard for heavy metal Pb for marine biota is 0.008 ppm. Acute Pb toxicity in the waters can damage the kidneys, the reproductive system, the liver, the brain, the central nervous system, and can cause death (Jaishankar et al 2014).

Considering the danger of Pb to living organism, its presence in the environment especially in waters must be minimized. One way to reduce the metal content of Pb in aquatic mediums is to use biofilters, which is a method carried out by utilizing living organisms aimed at reducing pollution in aquatic environments containing toxic materials (Lim et al 2015). The method is applied by utilizing a type of macroalgae in order to absorb the dissolved metal in waters.

Gracilaria verrucosa is one of themacroalgaes of the Rhodophyta division that grows in the tropics and subtropics (Sjafrie 1990). This seaweed has a high absorption ability of heavy metals because its cell wall contains polysaccharides (Handhani et al 2017; Yong et al 2017). The polysaccharide contained in this red seaweed is the agar. Agar is formed from a mixture of two polysaccharides, i.e. agarose and agaropectin (Phillips & Peter 2000). The accumulation of heavy metals occurs because the polysaccharides present in *G. verrucosa* cell wall can bind heavy metal ions and form complex compounds with organic substances contained in the thalus (Lobban & Harrison 1994). Study on the effectiveness and efficiency of *G. verrucosa* as a biofilter to reduce heavy metal content of lead (Pb) on the sea water media is important to be carried out. The purpose of this study was to determine the percentage of effective absorption rate of Pb by red seaweed *G. verrucosa* at various period of exposure.

Material and Method. The red seaweed *G. verrucosa* was obtained from seaweed culture ponds of Jepara, Central Java. Only fresh, no white patches, not pealing, and elastic seaweeds were selected (Akmal et al 2008). The experimental media pollutant solution used was concentrated Pb acetate (Pb(CH3COO)2.3H2O) which was previously made into a stock solution with a concentration of 1,000 ppm.

This research used a Completely Randomized Design with 2 factorials, each factor was repeated 3 times. First factor was Pb concentration, namely: 0.00, 0.06, 0.60 and 6.00 ppm (P1 to P4) according to Ologuin et al (2009), Nasuha et al (2014) and Ihsan et al (2015). The second factor was the exposure time as: 7, 14, 21, 28 days. The acclimatization was done by incubating seaweed in sea water media at salinity of 25 ppt for 7 days (Mosquera & Salamanca 2016). Seaweeds were distributed in each aquarium at weight of ±200 g. Seaweeds were divided into two bonds, 50 g for growth measurements and 150 g for samples to be analyzed for their absorption of Pb metal. To increase the growth of *G. verrucosa*, 25 g NPK fertilizer was added to media in each aquarium (Alamsjah et al 2009).

Twelve clear glass aquariums with volume of 50 x 30 x 35 cm were used (Yulianto et al 2006). Each aquarium was equipped with an aeration pump placed in the center, and filled with 25 liters of sea water at 25 ppt salinity. Before culture media was used, Pb concentration was measured as control. Pb acetate was added in the seawater media according to the treatment. A 40 watt TL lamp equivalent to 3500-4000 lux was used in this experiment (Alamsjah et al 2009). The maximum light intensity for *Gracilaria* sp. was 4750 lux (Dawes 1981). Photoperiod was adjusted so as to natural condition at 12 hours light and 12 hours dark.

The growth of seaweed was measured in wet weight basis every week for 28 days as well as for the condition of seaweed. Water quality measurement in culture media such as temperature, salinity, and pH was carried out daily at 10:00 AM. Meanwhile, Pb concentration was analyzed every week by using Atomic Absorption Spectrophotometer.

Absorption ability of seaweed to Pb was determined by calculating the absorption percentage and absorption capacity based on formula used by Rahman & Sathasivam (2015). The data obtained were analyzed statistically using Anova test. All data were tested for normality and homogeneity prior to statistical analysis. Regression tests were also conducted to determine the relationship between treatments

Results and Discussion. The results showed that the addition of different concentrations of Pb at different time of exposure affected on heavy metal concentration in seaweed, sea water medium, effectiveness and efficiency of Pb absorption and wet weight. Time of exposure showed significant changes to Pb concentration in *G. verrucosa*, culture media, effectiveness and efficiency, and growth of seaweed ($P<0.05$), while treatment of Pb did not showed any differences.

Reduction of Pb. Time of exposure significantly ($P<0.05$) decrease Pb concentration in seawater media (Figure 1). T0 exposure time was found significantly different from other treatments. The average decrease in daily Pb concentration in the sea water medium at P1 was 0.013 ppm, at P2 was 0.015 ppm, at P3 was 0.018 ppm, and at P4 was 0.017 ppm.

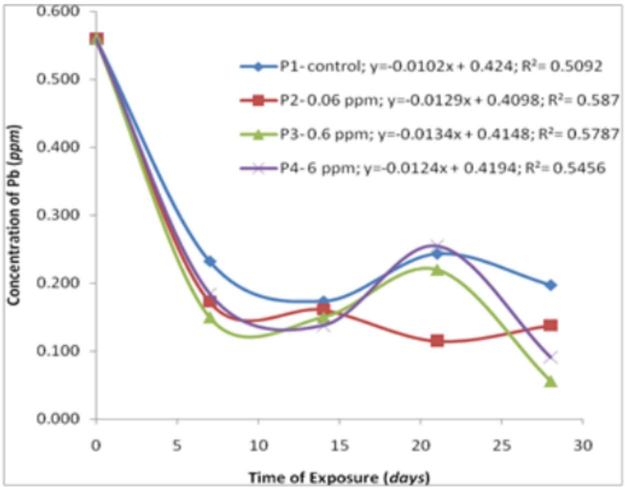

Figure 1. Concentration of Pb in seaweed culture media (seawater) after 28 days period of exposure at various Pb Concentrations (P1: control without Pb addition; P2: Pb addition of 0.06 ppm; P3: Pb addition of 0.6 ppm; and P4: Pb addition of 6 ppm).

The decrease of Pb in the media indicated that seaweed has the ability to adsorb the heavy metals. The transfer of Pb from the seawater into seaweed was predicted due to variation of concentration. The high concentration of Pb in the seawater diffused into lower concentration of Pb in seaweed tissue. According Raya & Ramlah (2012), the concentration of heavy metals in culture media affected the amount of heavy metals accumulated by seaweed.

Pb content on seaweed. The results revealed that Pb concentration in seaweed increased in each treatment during the 28 days of exposure period (Figure 2). The absorption of Pb was affected by variation of Pb concentration in culture media and also by the time of exposure ($P<0.05$). The average increase of Pb concentration in seaweed per day for P1 was 0.002 ppm, for P2 0.372 ppm, for P3 0.521 ppm, and for P4 7.553 ppm. It was observed that at the first 7 days of culture the concentration of Pb in seaweed increased in all treatments and the highest increase was recorded in P4 treatment. This indicated that the ability of seaweed to absorb Pb was good and the condition at that time was not saturated, so Pb absorption was considerably fast, followed by the decrease until the 28th day indicating that Pb metal was close to a saturated state, except on the treatment of 0.6 ppm Pb concentration, where was a slight increase in heavy metal concentration. This was in accordance with Rahman & Sathasivam (2015) who stated that the capacity of biomass in heavy metal adsorption was increasing as the initial concentration of metal ions increased.

The process of Pb uptake by *G. verrucosa* was presumed to be influenced by the form of the thallus. According to Angkasa et al (2011), the shape of *G. verrucosa* thallus was cylindrical with a diameter ranging from 0.5 to 4.0 mm and forming a clump with an irregular branching type. Nduka (2012), stated that materials which have smaller diameter have higher absorption rates than larger diameter adsorbents. Thallus diameter of G. verrucosa is very small and shaped like a fiber that supposedly facilitates in interacting with the substances contained in the solution thereby increasing the ability to absorb Pb. The seaweed was able to accumulate Pb through the surface of the thallus cells and spread it throughout its organs to the medulla part (Stengel et al 2005). This was proved by the decreasing concentration of Pb in culture medium in the present study.

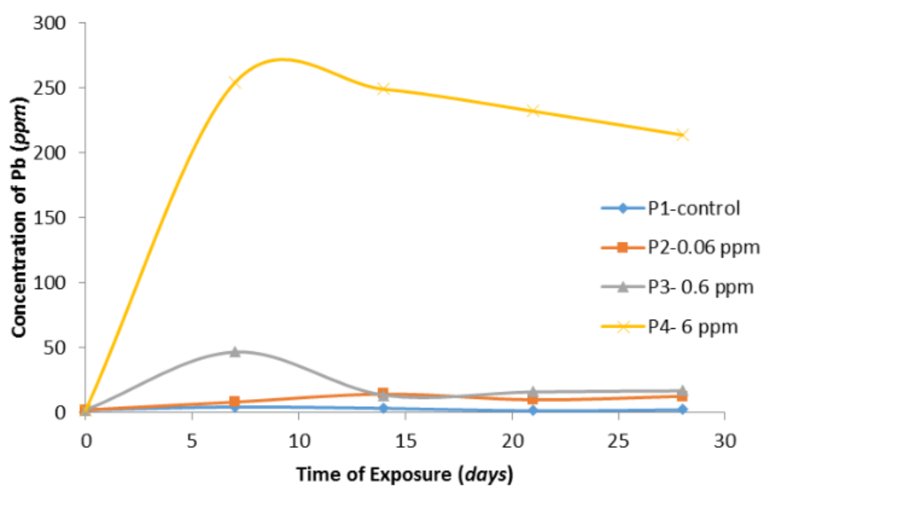

Figure 2. Concentration of Pb in seaweed *Gracilaria verrucosa* cultured in various Pb contained media (P1: control without Pb addition; P2: Pb addition of 0.06 ppm; P3: Pb addition of 0.6 ppm; and P4: Pb addition of 6 ppm).

***Absorption capacity and efficiency of seaweed**.* Capacity of Pb metal uptake by seaweed *G. verrucosa* against exposure period is presented in Figure 3.

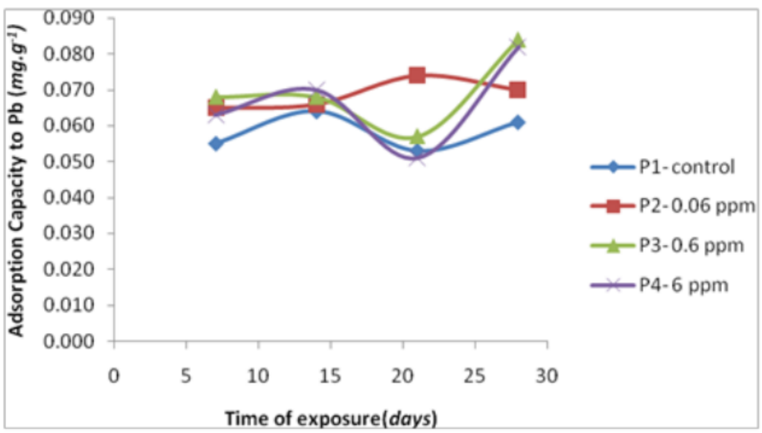

Figure 3. Absorption of Pb by red seaweed *Gracilaria verrucosa* function of time exposure (P1: control media without Pb addition; P2: Pb added media 0.06 ppm; P3: Pb added media 0.60 ppm; P4: Pb added media 6.00 ppm).

This process was carried out at an initial Pb concentration of 0.560 mg.L^{-1} and exposure period of 7, 14, 21, and 28 days. Figure 4 revealed that compared to others treatments the absorption rate from T7 to T28 was the highest (at Pb concentration of 0.6 ppm) by 0.068, 0.068, 0.057, and 0.084 mg.g^{-1} and the percentage of absorption reached 73.28%, 73.28%, 60.72% and 75.28%. It can be concluded that the highest Pb absorption ability of *G. verrucosa* occurred at the 0.6 ppm Pb concentration, where in this condition the seaweed was not saturated, thus increasing the absorbed Pb. In contrast, the treatment with 6.00 ppm Pb concentration resulted a low absorption. According to Bhattacharyya & Gupta (2007), at the beginning of absorption, the active site on the surface of the adsorbent was fully open to Pb (II). This caused more Pb^{+2} to be absorbed on the surface of the adsorbent, after the surface of the adsorbent reached to saturated with Pb^{+2}, there was no increase in its absorption capacity.

The absorption capacity of *G. verrucosa* toward Pb was thought to be due to the presence of cell wall polysaccharide content which can bind heavy metal ions and form complex compounds with organic substances contained in the thallus (Lobban & Horrison 1994).

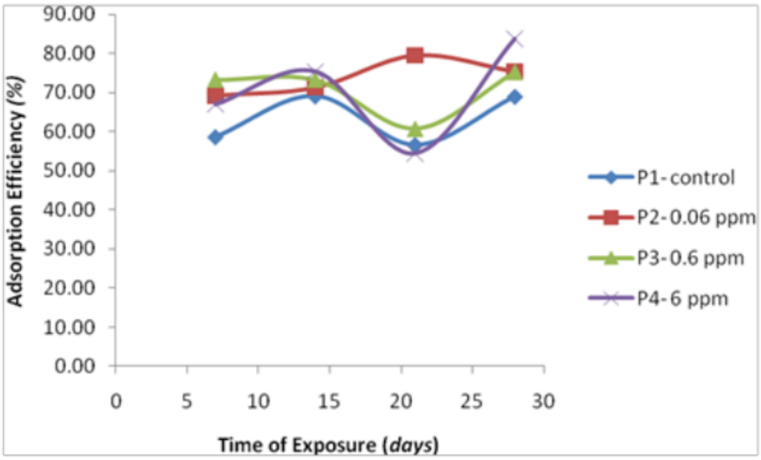

Figure 4. Absorption efficiency to heavy metal Pb by red seaweed *Gracilaria verrucosa* as a function of exposure time (P1: control without Pb addition; P2: Pb addition of 0.06 ppm; P3: Pb addition of 0.6 ppm; and P4: Pb addition of 6 ppm).

Growth of G. verrucosa. Seaweed grew at all Pb concentrations culture media. The highest growth was achieved at P4 (6.00 ppm addition of Pb). Weekly growth was recorded as 50.07, 57.79, 60.71, 64.31, and 64.03 g $week^{-1}$ with an average daily growth of 0.50 g day^{-1}. The lowest growth, however, was recorded at P1 treatment (control) at wet weight increase from T0 to T28 of 50.06, 55.99, 55.80, 58.72, and 57.31 g $week^{-1}$ with an average daily growth of 0.26 g day^{-1} (Figure 5).

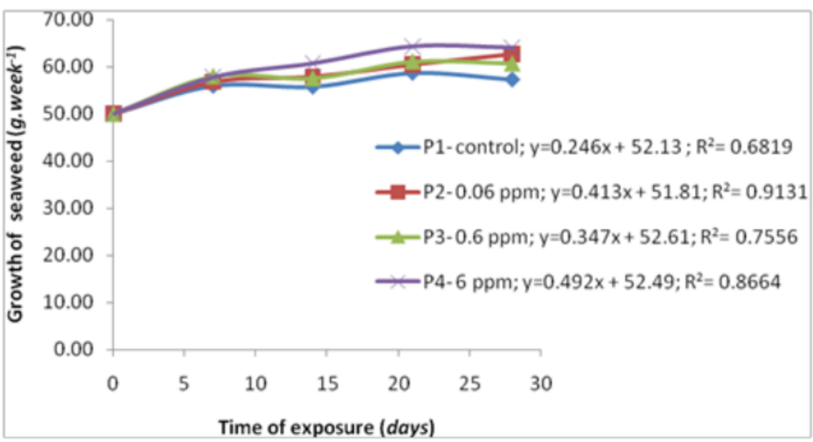

Figure 5. Growth of red seaweed *Gracilaria verrucosa* cultured for 28 days period under different concentrations of Pb (P1: control without Pb addition; P2: Pb addition of 0.06 ppm; P3: Pb addition of 0.60 ppm; and P4: Pb addition of 6.00 ppm).

The high wet weight of seaweed on P4 treatment was in line with the high percentage of Pb uptake by seaweed up to 83.69%, but inversely with the seaweed absorption capacity of Pb. The highest absorption capacity of Pb of *G. verrucosa* was found in 0.6 ppm Pb (P3) with 0.084 mg g^{-1}. The high percentage of Pb absorption by seaweed was suspected to affect the concentration of heavy metals contained in the thallus of seaweed. Continuous absorption reached saturation so that there would be clumping of the adsorbent so that the surface was not entirely open. This results in reduced active surface area of the adsorbent (thallus) resulting in ineffective absorption process and in reduced absorption capacity. According to Nurafriyanti et al (2017), if the surface was saturated or closed to saturation it would undergo two processes. Firstly, a second absorption layer on top of the absorbent were bonded on the surface, this phenomenon was called multilayer absorption. Secondly, the second layer and so on is not formed so the adsorbent that has not adsorbed diffuses out of the pore and returns to the fluid.

Conclusions. The highest absorption ability of *G. verrucosa* toward Pb occurred at the concentration of 0.60 ppm media at 0.084 mg g^{-1} and absorption efficiency was equal to 75.28%. Meanwhile, the lowest absorption ability occurred at control (no Pb media) equal with 0.061 mg g^{-1} with absorption efficiency amounted 68.93%. The adsorbent saturation process was recorded at 6.00 ppm Pb added media with absorption capacity of 0.082 mg g^{-1} and efficiency of 83.69%.

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