Reduction of Pb concentration in seawater by seaweed *Gracilaria verrucosa*

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Abstract. Heavy metal pollution, particularly Pb, may damage estuary and coastal aquatic ecosystems. It could reduce the water quality. Seaweed, *Gracilaria verrucosa*, is known of having an ability to absorb Pb in the water. This study aims to determine the ability of *G. verrucosa* in reducing the concentration of dissolved Pb in the water. Filtered seawater were added with different concentration of Pb, i.e. P1 as a control (without the addition of Pb), P2 (3 ppm of Pb), P3 (6 ppm of Pb) and P4 (11 ppm of Pb). All treatments were done in triplicate. The concentrations of Pb in the seawater and the seaweed were measured by using AAS (Atomic Absorption Spectrophotometry) at 0 time (before Pb addition), 5, 10, and 15 days after Pb addition. The average value of the decrease of Pb concentrations were 0.016, 0.125, 0.21, and 0.32 mg L^{-1} day^{-1} for P1, P2, P3 and P4, respectively. The concentrations in the seawater are inversely proportional to the average increase of the heavy metal concentrations in *G. verrucosa* per day. The values were 0.28, 1.79, 2.98, and 4.67 mg L^{-1} for P1, P2, P3 and P4, respectively. The results suggest that the higher of Pb concentration and the longer of exposure, the more heavy metal that can be absorbed in *G. verrucosa* thus improving the water quality.

Key Words: marine pollution, heavy metal, biosorption, human health.

Introduction. Estuary and coastal aquatic ecosystems are among the areas vulnerable to pollution due to the adjacent location to human activities on the mainland (Arifin & Fadhlina 2010; Wang et al 2013; Jazza et al 2016). According to Amrizal (1991), ocean is the final spot of various wastes of human activities which lead to decreasing seawater quality. Consequently, the ecosystem is likely to be a waste build up of the activities along the coast as well as the upstream. Inorganic waste and heavy metals entering the water are hard to degrade and having a high level of toxicity compared to pesticides (Palar 2008). Heavy metals contained in the water are derived from erosion process, industrial waste, domestic waste and agricultural activities (Etim et al 1991; Suryono et al 2007; Purwiyanto 2015). Heavy metal pollution that damages the aquatic ecosystem of ecological aspect is determined by the level and continuity of pollutants entering the water, bioaccumulation and toxicity properties (Ambariyanto 2011). Heavy metal Pb is a non-essential metal, in which its presence is toxic to organisms in the water (Achmad 2004).

This kind of pollution will not only affect marine organisms (Ambariyanto 2017), but also human health. WHO HECA (2002) stated that the virulence of heavy metal Pb may give adverse impacts to human, such as causing kidney malfunction, inhibited growth, decreased IQ and death. The Pb concentration of 188 ppm can kill fish, and the concentration of 2.75 to 49 ppm after 245 hours will cause death for Crustaceans (Palar 2008). Acute toxicity of Pb in the water may damage kidneys, reproductive system, liver, brain, central nervous system, and death in humans (Achmad 2004).

Heavy metal content in the water can be reduced by biofilter which is known as a method that utilizes living organisms to reduce the amount of toxic pollutants in the water (Yulianto et al 2006). Marine macro algae were suggested to be an inexpensive and reliable method to reduce heavy mental (Sweetly et al 2014) as well as bioindicator of the heavy metal pollution in seawater (Al-Homaidan et al 2011). They have an ability to absorb heavy metal ions due to their functional groups contained in the outer and inner surfaces of algae cells that bind to metal ions (Elfrida 2009). Several species of red seaweed widely used as biofilter include *Eucheuma* sp., *Gelidium* sp., *Gracilaria* sp., and *Hypnea* sp. It is also known that various species of algae, especially green algae (Chlorophyta), brown algae (Phaeophyta), and red algae (Rhodophyta) can adsorb metal ions (Davis et al 2003; Raya & Ramlah 2012). A seaweed *Gracilaria verrucosa* has the ability as an alternative in the separation of trace elements such as dangerous heavy metals with high biosorption capacity (Ariyanti & Nurcahyani 2012). This species is widely distributed in most of Indonesian coastal areas (Sulistijo 1985). This study aims to determine the ability of seaweed *G. verrucosa* in reducing the concentration of heavy metal Pb dissolved in seawater and the concentration of heavy metal Pb which can be optimally absorbed by this seaweed.

Material and Method. This research was done on October-December 2016. A stock solution of lead metal (Pb) was derived from $Pb(CH_3OO)_2$. This stock solution was made by weighing 1.4493 g of $Pb(CH_3OO)_2$ which was then dissolved in 1 liter of distilled water to provide 1000 mg L^{-1} of Pb solution. Afterwards, it was diluted in accordance with the required concentrations treatment. In this study, one control and three treatments with three replications were conducted, i.e. P1 (control; no Pb addition), P2 (3 ppm), P3 (6 ppm) and P4 (11 ppm).

The test containers (36 plastic jars with a volume of 12 L) were divided into four groups representing control and treatments. Each container was filled with 10 liter of filtered seawater. Seaweed, *G. verrucosa* (100 g) which was obtained from Jepara, Central Java coastal area was put in each jar. The oxygen levels in the growing media were maintained by using aerators in each aquarium. Irradiation of *G. verrucosa* was obtained from TL lamp with light intensity 400 lux (Aslan 2006).

The concentrations of heavy metal Pb were measured by using Atomic Absorption Spectrophotometer (AAS) within the seawater and the seaweed. Measurements were done at 0 days (T0), 5 days (T5), 10 days (T10) and 15 days (T15) after Pb addition. The measurements of seawater parameters i.e. salinity, pH, dissolved oxygen (DO) and temperature were done every day at 11 am.

Data on Pb content within the water and seaweed were analysed by using univariate test to determine whether there were effects among different treatments. Normality test was performed prior to univariate test to determine whether the data were normal. Regression test was carried out to obtain the relationship among the reduction of Pb in the seawater, Pb absorbed into the seaweed, and the growth of seaweed *G. verrucosa* for 15 days.

Results and Discussion

Reduction of Pb. Figure 1 shows that for 15 days, P4 has an average difference value which is quite high compared to P1, P2 and P3. The average of the decrease value of Pb concentrations per day in the medium of seawater on P1 is 0.016 mg L^{-1}, on P2 is 0.125 mg L^{-1}, on P3 is 0.21 mg L^{-1} and on P4 is 0.32 mg L^{-1}.

Univariate test in the seawater with the different concentrations of Pb and different periods planted by seaweed *G. verrucosa* shows obvious different effects. It is indicated by the value in the addition of Pb concentration ($p < 0.05$), period ($p < 0.05$) as well as the interaction between the periods and the addition of Pb ($p < 0.05$), which indicates there are different effects among the ability of seaweed *G. verrucosa* in reducing heavy metal Pb in the seawater and the different concentrations of heavy metal Pb, different timescales and the interaction between the periods and the addition of Pb.

The higher the concentrations of Pb applied, the higher the rate of the reduction of Pb contained in the seawater. Different concentrations between the seawater and the seaweed tissue cause diffusion displacement. Therefore, the concentration of Pb in the seawater allegedly breaks into the seaweed tissue that has lower concentration. The heavy metal concentration in the maintenance media will affect the amount of heavy metal accumulated by the seaweed (Raya & Ramlah 2012).

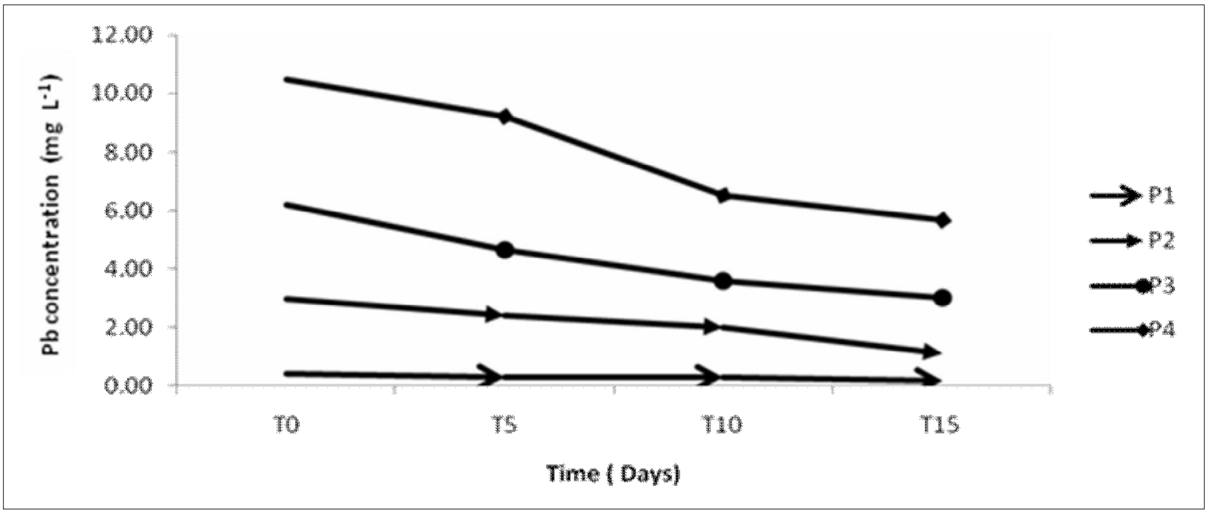

Figure 1. Concentrations of Pb in the seawater (P1 as a control without the addition of Pb; P2= 3 mg L^{-1}; P3= 6 mg L^{-1}; P4= 11 mg L^{-1}).

***Pb content in G. verrucosa**.* The results of reducing the concentration of Pb in the seawater are inversely proportional to the concentration of the seaweed *G. verrucosa*. The concentrations of the seaweed are likely to increase in each treatment for 15 days of growing period. The measurement results of the absorption of Pb by *G. verrucosa* is presented in Figure 2*.*

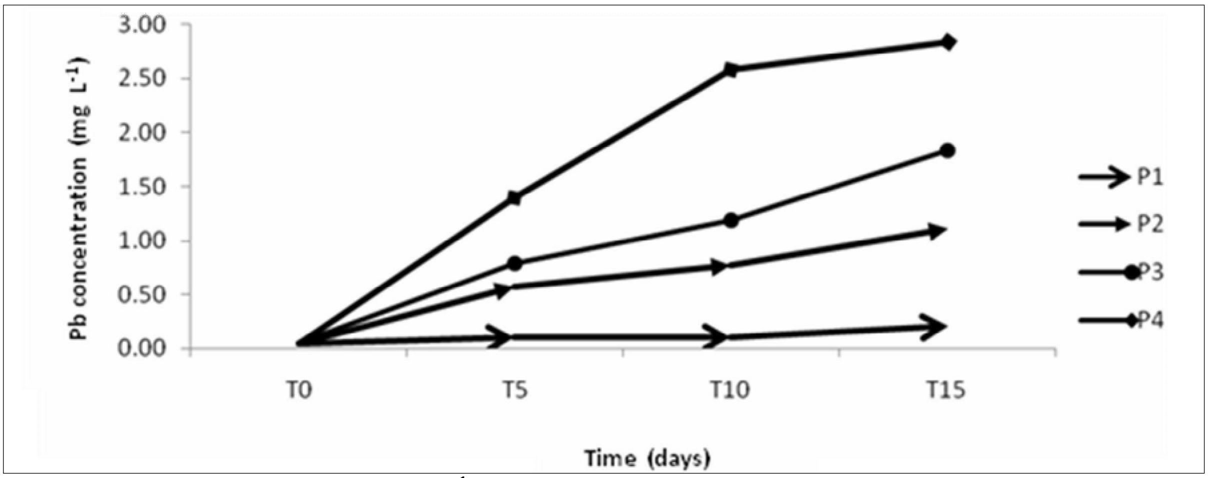

Figure 2. Concentrations of Pb (mg L^{-1}) in seaweed *G. verrucosa* planted in the medium of seawater with different concentrations of Pb (P1 as a control without the addition of Pb concentration, P2 with the addition of 3 mg L^{-1} concentration, P3 with the addition of 6 mg L^{-1} concentration, and P4 with the addition of 11 mg L^{-1} concentration).

The results show that for 15 days, P4 has an average difference value of the increase of heavy metal concentration that is considerably higher than P1, P2 and P3. The average of the increase of heavy metal concentration in *G. verrucosa* per day on P1 is ±0.01 mg L^{-1}, on P2 is ±0.07 mg L^{-1}, on P3 is ±0.12 mg L^{-1} and on P4 is ±0.19 mg L^{-1}.

Univariate test analysis on seaweed planted in seawater by adding different concentrations of Pb also has significant different effects shown by the value of the addition of Pb ($p < 0.05$), period ($p < 0.05$) and interaction between the periods and the addition of Pb ($p < 0.05$). It shows that the addition of different concentrations of Pb, different periods, and the interaction between the periods and the addition of Pb in each seawater with the concentration of Pb planted by *G. verrucosa* affects the ability of *G. verrucosa* to absorb Pb, in which the higher the concentrations of Pb, the more Pb absorbed by *G. verrucosa*.

Different concentrations of Pb absorbed in the medium of seawater and those absorbed by *G. verrucosa* might happen because of the microorganisms, phytoplankton or other cyanobacteria in the medium of seawater that absorbs Pb or uses Pb for its metabolic process. Pb can form a bond with organic ligand containing N, O and S which acts as the donor (Darmono 1995). Pb concentration in the water can be adsorbed by using phytoplankton such as *Chlorella* sp. (Soeprobowati & Hariyati 2012); *Spirulina* sp. (Chen & Pan 2005) and *Chlorella vulgaris* (Purnamawati et al 2015).

Parameters of seawater quality. Differences in seawater parameters such as pH, salinity and DO will influence toxicity level of heavy mental. Lower pH value will cause higher toxicity of Pb which may cause the higher accumulation level of Pb in the organism (Hutagalung 1991). Salinity in the seawater which rises will lead Pb ions to bind to other molecules, and the ions of Pb will decrease in the seawater. Changes in DO value in the medium of seawater also affect the toxicity level of Pb, where the lower of DO value will cause higher the toxicity level (Palar 2008). Our results showed that the values of seawater parameters measured during the experiments were very similar between treatments (Table 1). Therefore, it is believed that the influence of water quality during the experiment on the absorption of PB by seaweed were similar at all treatments.

Table 1

Parameters of seawater quality during the experiment

No	*Seawater parameters*	*Treatments*			
		P1	*P2*	*P3*	*P4*
1	pH	6.87±0.07	6.85±0.04	6.86±0.04	6.83±0.01
2	Temperature (^{o}C)	29.32±0.13	29.18±0.04	29.14±0.05	29.12±0.09
3	Salinity ($^{o}/_{oo}$)	31.08±0.81	29.46±0.05	30.30±0.66	30.50±0.66
4	DO (mg L^{-1})	5.07±0.26	5.00±0.10	5.54±0.32	4.46±0.08

Growth rate of G. verrucosa. The growth of seaweed *G. verrucosa* living in seawater at different concentrations of Pb for 15 days is represented in Figure 3. Each addition of different concentrations of Pb in each media causes different growth effects at the end of cultivation. The growth of *G. verrucosa* can be calculated from the wet weight gains at each treatment. The wet weight gains of *G. verrucosa* for 15 days are 25 g on P1, 26.67 g on P2, 30 g on P3, and 40.33 g on P4.

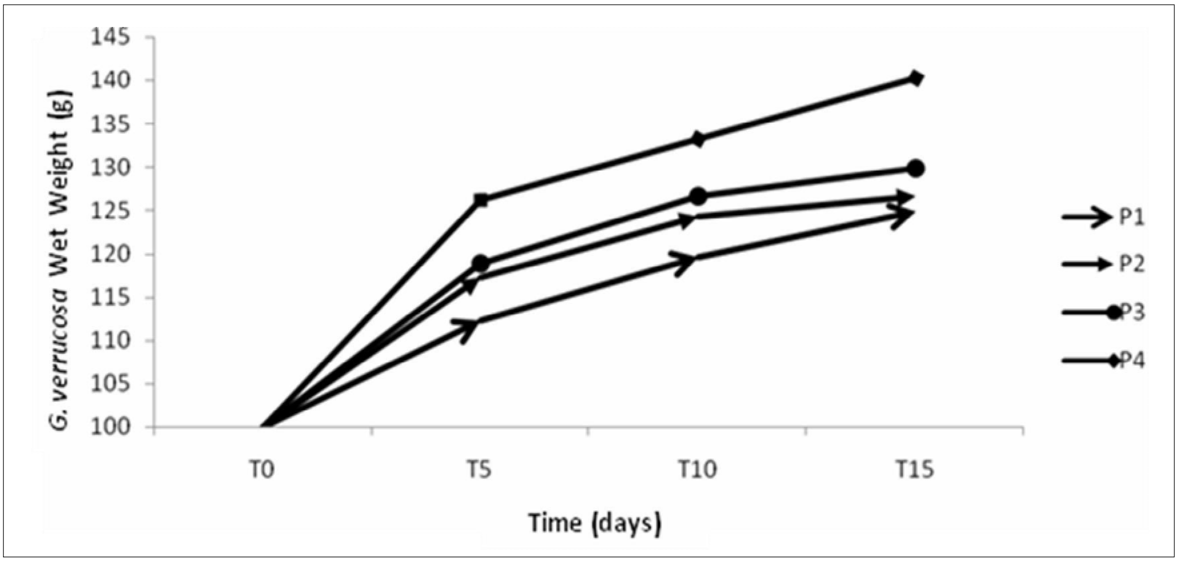

Figure 3. Growth of *G. verrucosa* in the seawater with different concentrations of Pb (P1 as a control without the addition of Pb concentration, P2 with the addition of 3 mg L^{-1} concentration, P3 with the addition of 6 mg L^{-1} concentration, and P4 with the addition of 11 mg L^{-1} concentration) for 15 days.

The relation between the addition of different concentrations of Pb in the seawater and the growth rate of seaweed *G. verrucosa* is summarized in Figure 4 with an equation $y = -0.345x^2 + 6.511x + 100.6$ and the value of $R^2 = 0.879$.

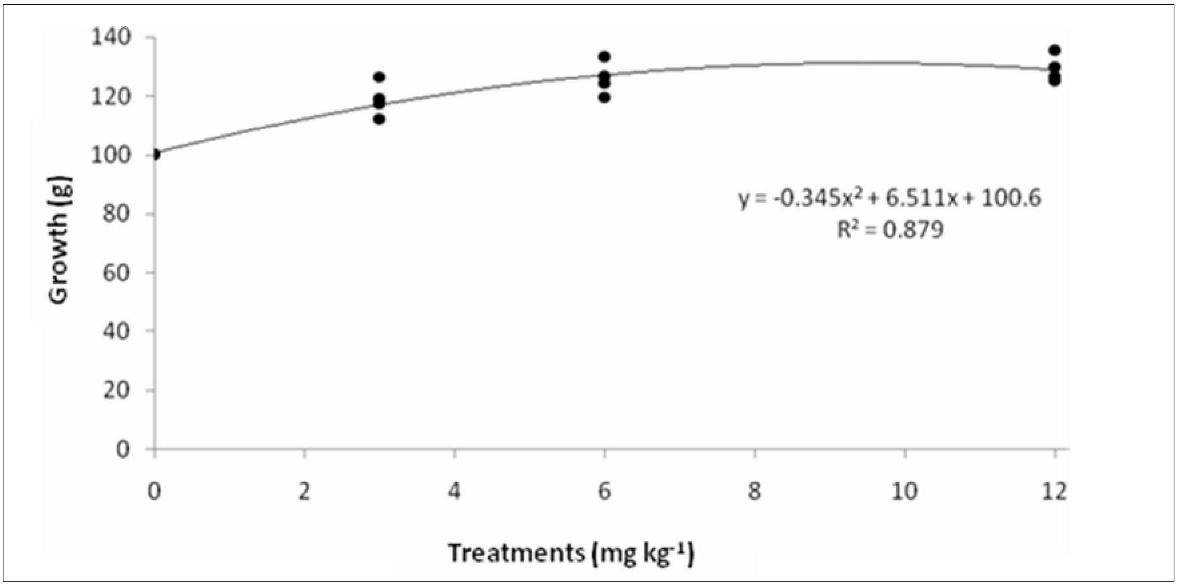

Figure 4. Relationship between the growth of *G. verrucosa* in the seawater and different concentrations of Pb (P1 as a control without the addition of Pb concentration, P2 with the addition of 3 mg L^{-1} concentration, P3 with the addition of 6 mg L^{-1} concentration, and P4 with the addition of 11 mg L^{-1} concentration).

Accumulation of Pb occurs because the Pb, together with organic substances contained in the talus of the seaweed form complex compounds so that the Pb is fixed within the talus and can not be excreted (Lobban & Harrison 1994). Plants that live in polluted environments have coping mechanisms that cause the pollutants to be active then be stored in the tissue. Heavy metals that are stored in the vacuole would lead to the vacuole expand thereby increasing the weight of the plants (Lobban & Harrison 1994).

The results show that the seaweed *G. verrucosa* has the potential to reduce pollutants, especially Pb in seawater. Seaweed *G. verrucosa* has fairly good ability to absorb heavy metal pollutants. In addition, this seaweed also has a high survival rate in Pb polluted seawater. This potential can be used to increase water quality with its ability and resilience in reducing heavy metal in polluted water.

Conclusions. The ability of seaweed *G. verrucosa* to reduce heavy metal Pb with different concentrations in the seawater is influenced by concentration and expose period. The higher the concentration and longer expose of Pb, the higher the heavy metals that can be reduced in seawater and thus improving the water quality. The highest average value of the decrease of Pb concentrations was 0.32 mg L^{-1} day^{-1}. While the average increase of the heavy metal concentrations in *G. verrucosa* per day were 0.28, 1.79, 2.98, and 4.67 mg L^{-1} for P1, P2, P3 and P4, respectively.

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