Preliminary study on the potential of *Sargassum* macroalgae as lead (Pb) biosorbent agents

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Abstract. Heavy metal pollution in waters is increasingly spreading. It can cause water quality degradation that could affect the life of different organisms. One effort to reduce the concentration of heavy metals in waters is the use of macroalgae as biosorbent. This study aims to analyze the ability of *Sargassum* in living and dry conditions as a sorbent of lead [$Pb(NO_3)_2$] at various concentration levels, namely 0 (control), 0.5, 1 and 1.5 ppm. The results indicated that *Sargassum* has a high performance as $Pb(NO_3)_2$ biosorbent agent. The $Pb(NO_3)_2$ sorption can reach 100% within 96 hours for live *Sargassum*, and 98% within 15 hours for dry *Sargassum*. Other results indicated that a decrease in the concentration of $Pb(NO_3)_2$ in dry *Sargassum*, after going through the process of immersion and stirring for 15 hours in water without $Pb(NO_3)_2$ content, shows that $Pb(NO_3)_2$ content in *Sargassum* can be released again. *Sargassum* in living and dry conditions are feasible be used as an inexpensive, practical and reusable biosorbent.

Key Words: cheap sorbent, dry *Sargassum*, heavy metals, live *Sargassum*, pollution.

Introduction. The rapid increase in human population causes an increase in the volume of organic material and metals that enter and pollute water and sediments, (Canbay et al 2010; Meyer-Reil & Köster 2000; Nasir et al 2016; Qiao et al 2013; Sany et al 2013; Wurl & Obbard 2004), mainly urban and industrial waste. Industrial development has an impact on increasing the solid, liquid, and gas waste volume. Some of these wastes contain toxic and dangerous chemical substances, such as heavy metals (Lestari & Edward 2004). Heavy metals are one of the compounds dominant in water pollution (Yantyana et al 2018). Heavy metals are widely used by industries as raw materials, catalysts and supporting materials (Suyanto et al 2010), which, when released into nature, are not only toxic, but can also accumulate in living organisms (Ashraf 2006; Hananingtyas 2017). Heavy metals need serious countermeasures because they can seriously degrade aquatic ecosystems.

Since the mercury poisoning case from 1953 in Minamata, Japan, was revealed (Putranto 2011), the occurrence of environmental pollution by heavy metals is increasingly being reported. Lead (Pb) is one of the heavy metals polluting marine and estuary waters, with the main source being industries and transportation (Batley & Gardner 1978; Boening 1999; Budiastuti et al 2016; Fabris et al 1994; Nur & Karneli 2015; Setiawan 2014). Pb poisoning can cause health problems in humans, such as disorders of the immune system, nervous system, senses, kidneys, reproductive system, and digestive tract (de Freitas et al 2007; Laila & Shofwati 2013; Mishra 2009; Needleman 1991; Tong et al 2000; Wigg 2001).

Many efforts to minimize non-essential metals such as Pb in waters have been carried out with various methods, including the use of biomass as biosorbent. Biomass known to accumulate heavy metals is bacteria (Sag & Kutsal 1995; Scott & Karanjkar 1992; Stranberg et al 1981), fungus (Fourest et al 1994; Huang et al 1991; Matheickal et al 1997; Tobin et al 1984), yeast (Huang et al 1990; Matheickal & Yu 1996; Volesky et al 1993), peat (Breuer & Melzer 1990; Ferguson et al 1989), and algae (Chong & Volesky 1995; Fourest & Volesky 1996; Holan et al 1993). The use of biomass as a heavy metal adsorbent could be an appropriate alternative because in addition to the simple process, it is also environmentally friendly, and it can be reused (Gautam et al 2014; Lokeshwari & Joshi 2009; Ningsih et al 2016; Nursyamsi et al 2011; Rahmi & Sajidah 2017; Yantyana et al 2018; Zhang et al 2017; Zulkali et al 2006). The use of biomass, which can be abundant and easily found in nature, can reduce the operational costs of activities when compared to adsorption using synthetic ion exchangers (Fourest & Volesky 1996).

Macroalgae can adsorb pollutants in water, both as live macroalgae (Alim 2014; Ihsan et al 2015) and as dry macroalgae (Mahbub 2012; Nigro et al 2002; Yantyana et al 2018). Macroalgae can absorb pollutants because of the thallus with several functional groups such as hydroxyl, carboxyl, amino and sulfate, which can bind metal ions (Yantyana et al 2018). The entire surface of the macroalgae thallus can also absorb nutrients and other elements that are dissolved in water (Awaliah 2017; Nasuha 2014).

The use of macroalgae species as biosorbent agent must consider the socio-economic aspects. Macroalgae species that have been widely domesticated as food (Arbit et al 2019; Syamsuddin et al 2019), medicine and cosmetics (Ma et al 2019; Melanie et al 2020), and biofuels (Wadi et al 2019) may not be used as a biosorbent agents.

Sargassum is one of the potential macroalgae that can be used as biosorbent agent because it has not been widely domesticated. *Sargassum* is a brown macroalgae that has a very wide distribution in tropical waters, including in Indonesia. The thallus of this macroalga has the ability to form bonds selectively with metal cations of cadmium (Cd), copper (Cu), nickel (Ni), Pb, and zinc (Zn) (Fourest & Volesky 1996). The *Sargassum* cell wall is rich in polysaccharide content, such as alginic acid, which is composed of two monomers (β-D-manuronic acid and α-L-guluronate acid), and has a functional carboxylic acid group that can play an active role in binding metals (Yantyana et al 2018). Potential *Sargassum* as Pb biosorbent agent in both living and dry conditions still needs to be studied to obtain more information. In this study the ability of *Sargassum* to bind Pb ions in living and dry conditions is examined.

Material and Method. The research was carried out in October and November 2019. *Sargassum* sampling was done in the eastern part of Langkadea Island, Pangkep Regency, South Sulawesi, Indonesia (Figure 1a). Langkadea Island was chosen as a sampling location because it is an uninhabited island and is located far from the mainland of Sulawesi, and its waters are still relatively clean. *Sargassum* was abundant in these waters (Figure 1b and c). The measured environmental parameters were the pH, temperature, and salinity. They are measured using a digital multiparameter with an accuracy of 0.01ºC for the temperature and 0.01 ppt for the salinity.

Preparation, acclimatization and treatment. The research was carried out at the Multitrophic Research Group Laboratory, Hasanuddin University. The *Sargassum* collected from the sampling location (Figure 2a) was cleaned and washed thoroughly using seawater (Figure 2b). For live treatment, the clean *Sargassum* was acclimatized for three days in a one-ton tank filled with sterile seawater. Seawater was sterilized by using Sodium Hypochlorite (NaOCl) 1 ppm. Before seawater was used, NaOCl was neutralized by using thiosulfate solution ($Na_2S_2O_3$) 5 ppm. During acclimatization, the tank is provided with a circulating pump to move the water and increase the oxygen content in the water (Figure 2c). For dry treatment, clean *Sargassum* was air dried (with daily temperatures of 32-33ºC) for 2 days, then dried in an oven at 50^{0}C for 48 hours (Figure 2d).

Experimental design. The study used a Completely Randomized Design (CRD) with 4 levels of $Pb(NO_3)_2$ treatment: 0.5 mg L^{-1} (Treatment A); 1 mg L^{-1} (Treatment B); 1.5 mg L^{-1} (Treatment C); 0 mg L^{-1} (control). Each treatment had three replications. Lead was used in the form of $Pb(NO_3)_2$. The statistical tests used were the ANOVA test and the Tukey Test by using SPSS.

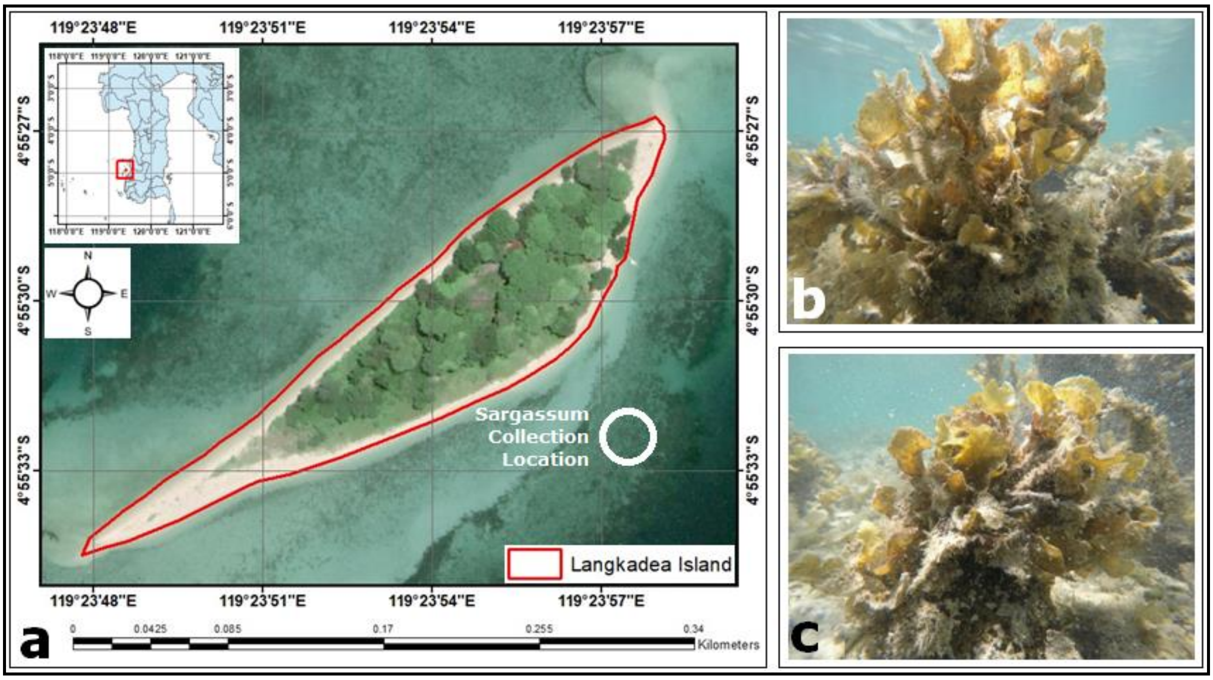

Figure 1. *Sargassum* sampling location (a) and *Sargassum* vegetation in the eastern part of Langkadea Island waters, Pangkep Regency, South Sulawesi, Indonesia (b and c).

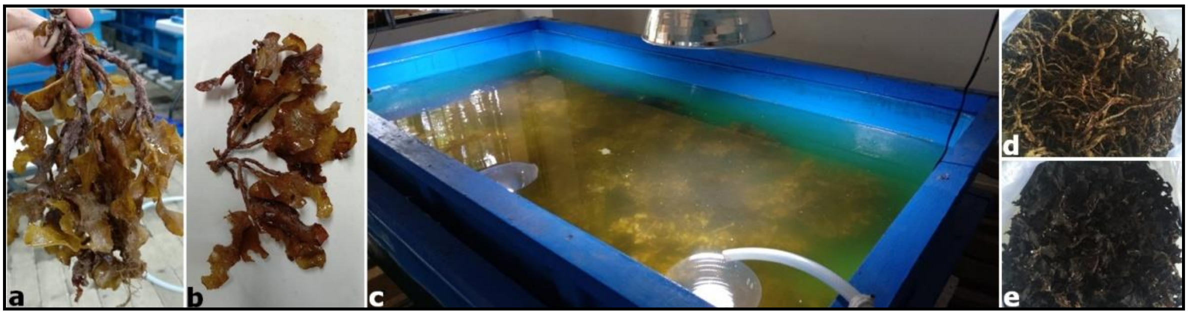

Figure 2. *Sargassum* condition before washing (a), after washing (b), acclimatization (c), dried stems (d), and dry leaves (e).

Metal exposure. Exposure of live *Sargassum* to $Pb(NO)_3$ (Figure 3a) was carried out by placing 100 g of live *Sargassum* into each treatment tank containing 17 L of seawater with $Pb(NO_3)_2$ concentrations according to each treatment (Figure 3b). Metal exposure was carried out for 4 x 24 hours. The duration of the study was limited to keep *Sargassum* fresh during the study, indicated by the physical conditions or by the appearance of the rigid texture of the thallus.

$Pb(NO_3)_2$ exposure for dried *Sargassum* was done by placing 10 g of dried *Sargassum* into plastic containers with 500 mL of seawater. $Pb(NO_3)_2$ was added according to the concentration of each treatment. During exposure (15 hours), the plastic containers were placed on a shaker with a stirring speed of 110 rpm (Figure 3c). Stirring was intended to provide an opportunity for all sorbents particles to come into contact with the sorbent compound (adsorbate). After the exposure was complete, 5 g of thallus were collected to analyze the $Pb(NO_3)_2$ content.

Alginate extraction. Alginate extraction from *Sargassum* was conducted after Mahbub (2012). Dried *Sargassum* was soaked in 1% HCl with a ratio between seaweed and HCl of 1:30 (w/v) and left for one hour. *Sargassum* was extracted using Na_2CO_3 (2%) at a ratio of 1:30, at 60-70°C for 60 minutes. The milling was done and the extraction was conducted again at a temperature of 60-70°C for 60 minutes. The mixture was filtered. The filtrate was bleached for 30 minutes by adding NaOCl, 4% of the filtrate volume. Alginic acid was formed by adding 10% HCl, with a pH between 2.8-3.2. After alginic acid was formed, it was washed to neutral using distilled water. With the addition of 10% NaOH (pH: 7-8), alginic acid was converted to Na-alginate, which was separated in isopropyl alcohol (IPA), 1:2 Na to IPA (v/v), while stirring evenly, after which the mixture was left for 30 minutes. The mixture (Na-alginate and IPA) was dried in an oven at 50ºC for 12 hours. The dried Na-alginate was grinded into alginate flour.

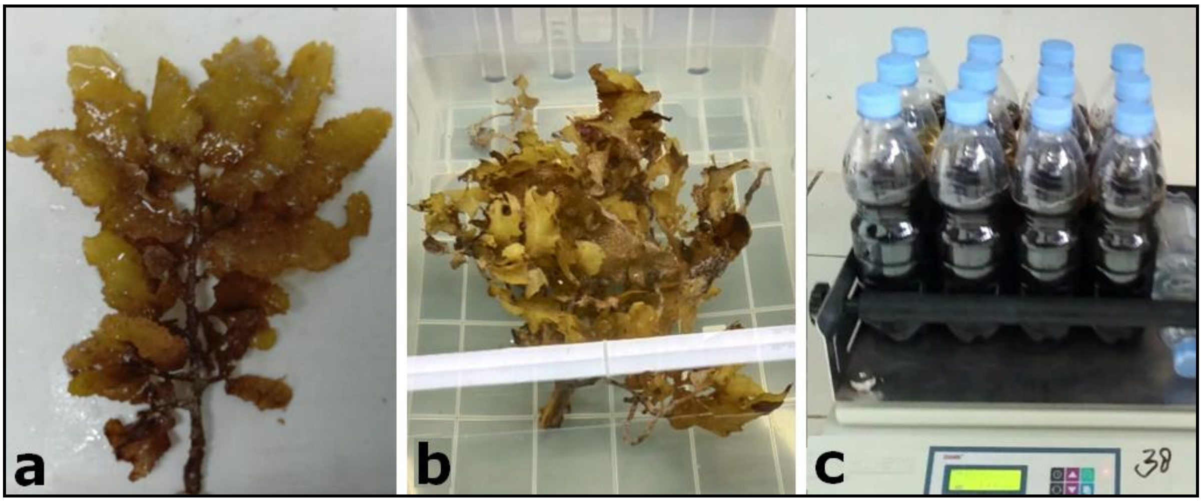

Figure 3. Fresh living *Sargassum* (a), living *Sargassum* treatment (b), and dry *Sargassum* treatment (c).

Dry Sargassum recovery. The recovery process was done by using *Sargassum* containing $Pb(NO_3)_2$. In the recovery process, *Sargassum* is immersed in the metal free seawater as the media. During the immersion process, stirring was carried out using a 110 rpm shaker. This recovery process, besides aiming to see the ability of *Sargassum* in releasing metal ions from its thallus, also aimed to determine the sorption process that occurs in *Sargassum* macroalgae in absorbing lead [$Pb(NO_3)_2$].

Results and Discussion. The phloid alginate content in the *Sargassum* leaves was 47.11±18.92%. This phloid alginate content is greater than that found in the *Sargassum* stem thallus, which is 15.67±7.84%.

$Pb(NO_3)_2$ sorption. The $Pb(NO_3)_2$ absorbed by living *Sargassum* from water at all concentration levels (0.5; 1.0 and 1.5 ppm) is 100%. For dry *Sargassum*, it was 98.0%; 97.7% and 98.67% (Table 1). After the statistical tests (ANOVA), lead sorption from water by living and dry *Sargassum* indicated that there were no significant differences among different concentration levels ($P > 0.05$).

Table 1

Percentage of $Pb(NO_3)_2$ sorption of *Sargassum*

Sargassum	*$Pb(NO_3)_2$ concentration (ppm) (Mean ± SE)*		*% Sorption (Mean ± SE)*
	Beginning of the treatment	*End of the treatment*	
Live	0	0	0
	0.5	0	100
	1	0	100
	1.5	0	100
Dry	0	0	0
	0.5	0.01	98
	1	0.023±0.009	97.7±0.882
	1.5	0.02±0.006	98.67±0.385

Effect of $Pb(NO_3)_2$ concentration. The t-test indicated that the sorption capacity between live and dry *Sargassum* was not significantly different (P>0.05). The average sorption of living *Sargassum* for each concentration level (0.0; 0.5; 1.0; and 1.5 ppm) were 0.367±0.015 ppm; 13.14±0.524 ppm; 15.837±0.352 ppm; and 15.46±1.446 ppm, respectively. The average sorption of dry *Sargassum* were 0.194±0.045 ppm, 12.107±1.904 ppm, 11.617±2.081 ppm, and 16.12±3.132 ppm, respectively (Table 2). The sorption values of live and dry *Sargassum* for different treatment concentrations of lead (excluding control) were not significantly different (P>0.05).

Table 2

Total $Pb(NO_3)_2$ sorption and desorption of *Sargassum*

Sargassum	*$Pb(NO_3)_2$ concentration (ppm)*	*$Pb(NO_3)_2$ concentration (ppm) in Sargassum*		*Total sorption (ppm) (Mean±SE)*	*Sorption (+) or desorption (-) (ppm) (Mean±SE)*
		Beginning of the treatment	*End of the treatment*		
Life	0	0.59	0.223±0.015	-0.367±0.015	0.367±0.015 (-)
	0.5	0.59	13.73±0.524	13.14±0.524	12.64±0.524 (+)
	1	0.59	16.427±0.352	15.837±0.352	14.837±0.352 (+)
	1.5	0.59	16.05±1.446	15.46±1.446	13.96±1.446 (+)
Dry	0	0.59	0.396±0.045	-0.194±0.045	0.194±0.045 (-)
	0.5	0.59	12.697±1.904	12.107±1.904	11.617±1.904 (+)
	1	0.59	12.207±2.081	11.617±2.081	10.64±2.073 (+)
	1.5	0.59	16.71±3.132	16.12±3.132	14.64±3.127 (+)

Recovery. The concentration of $Pb(NO_3)_2$ in dry *Sargassum* that has been used in previous treatments, after being immersed in water free of $Pb(NO_3)_2$, decreases from 18.014±0.502 ppm to 2.516±0.593 ppm, or by 15.498 ppm (86.03%). The $Pb(NO_3)_2$ concentration in seawater increases from 0.726±0.013 ppm to 0.8±0.015 ppm, or by 0.074 ppm (10.19%).

Water quality. During the study, the water quality was stable from the beginning to the end of the treatment, with a water pH value of 8, a temperature of 26ºC, and a salinity of 35 ppt (Table 3).

Table 3

Water quality parameters during treatment

Parameters	*Beginning of the treatment (Mean±SE)*	*End of the treatment (Mean±SE)*
pH	8.10±0.03	8.04±0.03
Temperature (^{0}C)	26.38±0.09	26.32±0.08
Salinity (ppt)	35.37±0.10	35.93±0.13

The $Pb(NO_3)_2$ content of *Sargassum* used in this study was 0.59 ppm. This concentration indicated that naturally, *Sargassum* actively absorbs Pb from its environment. This concentration is higher than the Pb natural content of marine animals, such as sea cucumber *Holothuria scabra*, which was only 0.05-0.07 ppm (Aprianto et al 2020). The safe limit of Pb concentration for human health is less than 2 ppm (SNI 2009).

Biosorbent agent. Alginate is the main component or important chemical compound in the cell walls of brown algae (Phaeophyceae). The Alginate content can reach 40% (Mahbub 2012; Ode 2014). Naturally, alginate plays a role in providing flexibility to marine algae in order to survive in ocean waters with currents and waves. Brown algae that live in more turbulent waters usually have higher alginate content compared to those that live in less turbulent waters (McHugh 2003). The higher alginate content of *Sargassum* in the leaves than in the stems may be due to the wider surface of the leaf causing it to receive greater hydrodynamic pressure than the stem, so the leaves must have more alginate content in order to be more flexible. The research of Mushollaeni & Rusdiana (2011) indicated that the number of leaves of the macroalgae is determined by the alginate. In the leaf, alginate fills the space between cells, so that it strengthens the leaf tissue (Sinurat & Kusumawati 2017). Alginate content in brown algae is influenced by species, climate and habitat conditions, such as light intensity, waves, currents, aquatic nutrients, etc. (Rasyid 2003; Sinurat & Marliani 2017).

Alginate plays a role in the formation of flexibility and the absorption properties of metals in macroalgae, in living and dead conditions. Some previous research results show that *Sargassum* powder is very good at absorbing Cd (Mahbub 2012). The research of Yantyana et al (2018) by using *Sargassum* extract in the form of Ca-alginate microcapsules shows that *Sargassum* is a Pb sorbent agent.

This study indicates that all $Pb(NO_3)_2$ particles contained in the water treatment media can be absorbed entirely by live *Sargassum* within 96 hours. The $Pb(NO_3)_2$ sorption capacity of *Sargassum* is related to the thallus, which has functional groups that can bind metal ions. These functional groups consist of carboxyl, hydroxyl, amine, sulfudril imadazole, sulfate and sulfonate groups (Alamsjah et al 2010; Chotimah et al 2016; Ibrahim et al 2012). The $Pb(NO_3)_2$ sorption capacity of *Sargassum* is also related to the cell wall, which is rich in polysaccharide content in the form of alginic acid, which is composed of two monomers, β-D-manuronic acid and α-L-guluronic acid, playing an active role in the metal binding process (Yantyana et al 2018).

Dry *Sargassum* has the ability to absorb metals. This research shows that dried *Sargassum* is able to absorb 98% of $Pb(NO_3)_2$ from seawater within 15 hours. In previous studies using Cd ions to characterize functional groups in the body of *S. crassifolium*, 5 functional groups were found, namely O-H, C-H, C=O, C-O-H and C-O. These five functional groups show differences in sorption intensity between before and after contact with Cd. The sorption intensity of the five functional groups decreased after contact with Cd, indicating that the clusters are bonded to the metal (Mahbub 2012).

The ability of live or dry macroalgae in absorbing metals is relevant to the previous studies. The previous studies indicate that macroalgae can naturally absorb metals effectively; for example *S. polycystum* was effective in absorbing Pb in water and sediments (Alim 2014) and other species of *Sargassum* can absorb Pb, Cd, Cr, Cu, and Mn (Manalu 2017). Some previous research shows the effectiveness of dry macroalgae as heavy metal adsorbents (Nigro & Van Staden 2002), but also the abilities of powdered macroalgae (Mahbub 2012), and Ca-alginate microcapsules (Yantyana et al 2018).

The results of this study indicate that live *Sargassum* was able to accumulate $Pb(NO_3)_2$ 10 to 26 times greater than the concentration of the Pb in the water. Dry *Sargassum* was able to accumulate 11 to 24 times greater concentrations of $Pb(NO_3)_2$ than what was found in the water. At a laboratory scale with the same concentration (1 ppm), marine animals can accumulate up to 53 times higher Pb levels (Yaqin et al 2014).

Desorption. The higher $Pb(NO_3)_2$ content in *Sargassum* (0.59) from the treatments than in the control (0 ppm) at the beginning of the study led to the desorption or release of lead metal ions from the sorbent (*Sargassum*). This desorption occurs chemically due to the breaking of chemical bonds that occur between the adsorbent and the adsorbate (Safitri 2016), when *Sargassum* is submerged in a medium with a lower $Pb(NO_3)_2$ content. Breaking this chemical bond causes the adsorbate to not be strongly bound to the adsorbent, so that it can be detached easily from the surface of the adsorbent, resulting in physical desorption (Safitri 2016).

Recovery. The use of macroalgae as heavy metal adsorbents can be considered as a simple solution, because the process is simple, environmentally friendly, and can be reused (Yantyana et al 2018). The recovery process is an effort to utilize waste (adsorbent that has been used) through the release of pollutant components contained in an adsorbent, so that the adsorbent can be reused (Mahbub 2012). The biomass recovery process depends on the sorption process that occurs in the adsorbent. In the sorption process, there are two terms, namely sorption and sorption. Both are distinguished by the gathering place of the substance absorbed. The sorption is the process of attaching molecules, ions, and atoms to the surface, while sorption is the process of the entry of liquid in the inner layer of the adsorbent (Handayani & Sulistiyono 2009). In the sorption process, the molecules are held freely on the surface and are easily released from the adsorbent, whereas for the sorption process, the molecules are dissolved or fully diffused in the absorbent to form a solution and cannot be easily separated from the absorbent (Handayani & Sulistiyono 2009). The decrease of Pb concentration in dry *Sargassum* after going through the process of immersion and stirring for 15 hours in seawater indicates that there has been a process of desorption or release of metal ions from the *Sargassum* thallus. This can be caused by breaking the bonds of metal ions with functional groups contained in *Sargassum* (Mahbub 2012).

Water quality parameters. Based on the results of previous studies, water quality parameters, such as pH, can affect functional groups that play an active role in biomass cell walls when absorbing heavy metals. Besides affecting functional groups, pH can also affect the solubility of metal ions in solutions (Yantyana et al 2018). The average water temperature during the study was slightly lower than the natural temperature in the waters (Ibrahim et al 2014), but on a microcosm scale, like the present study, it is normal for the water temperature to be lower (Tuwo et al 2019) because of less or no direct sunlight. Although water quality parameters can affect functional groups that play an active role in biomass cell walls when absorbing heavy metals, this could be overlooked in this study because all treatments have relatively similar water quality parameter values.

Conclusions. *Sargassum* has a high performance as $Pb(NO_3)_2$ biosorbent. $Pb(NO_3)_2$ sorption of *Sargassum* reached 100%, and 98% for dry *Sargassum*. The $Pb(NO_3)_2$ in the leaves and stems of *Sargassum* could be released. This study indicates that live and dry *Sargassum* can be used or reused as a cheap, practical biosorbent agent.

Acknowledgements. Thanks go to the Education Fund Management Institution (LPDP) of the Ministry of Finance of the Republic of Indonesia for scholarships to the first author, and Hasanuddin University for providing research and publication funding (contract number 1585/UN4.22/PT.01.03/2020 dated May 27th, 2020).

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Received: 27 April 2020. Accepted: 30 April 2020. Published online: 30 June 2020.
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How to cite this article:
Khatimah K., Yasir I., Tresnati J., Niartiningsih A., Yaqin K., Aprianto R., Tuwo A., 2020 Preliminary study on the potential of *Sargassum* macroalgae as lead (Pb) biosorbent agents. AACL Bioflux 13(3):1735-1745.