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Lead (Pb) Biosorption by Intact Biomass and Alginate Extract of *Sargassum crassifolium* Originated from Gresik Regency Waters

Faisol Mas'ud[1], Muhammad Browijoyo Santanumurti[2,3], Mohamed Hosny Gabr[3], Mamdouh T Jamal[3], Norma Aprilia Fanni[1], Moch Saad[1]*

[1]Department of Aquatic Resource Management, Faculty of Fisheries, Universitas Islam Lamongan
Jl. Veteran 53 A Lamongan Jawa Timur 12211, Indonesia
[2]Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga
Jl. Airlangga No.4 - 6, Airlangga, Surabaya, Jawa Timur 60115, Indonesia
[3]Department of Marine Biology, Faculty of Marine Sciences, King Abdulaziz University
Jeddah, 21589, Kingdom of Saudi Arabia
Email: muhammadsaad@unisla.ac.id

Abstract

The fisheries potential in Gresik Regency is in danger from industrial lead (Pb) pollution. One possible solution is using Sargassum crassifolium, which acts as an absorbent to eliminate Pb in waters. S. crassifolium is characterized by its great affinity for metal cations to bind heavy metal content. This study aimed at finding the effect of S. crassifolium in different forms (wet, dry, and alginate) to absorb Pb content in water. This research was conducted from July to September 2020. The water sample was taken from Gresik Regency and S. crassifolium was obtained from farmers in Talango Island, Sumenep District, Madura. The effectiveness test of the three treatments was conducted based on contact time and biomass factor. Based on the contact time, each treatment was compared with the contact time (1, 7, and 14 days) with 10 g mass per treatment. Meanwhile, for the biomass factor, each treatment was compared by weight (10, 20, and 30 g) for 7 days. The contact time showed that in 14 days, the alginate form of S. crassifolium had the highest absorbent power with 1.370±0.0034 mg.L^{-1} of lead absorbed and 100% absorbed value. Based on the absorbent mass treatment, 10 g alginate gave the best result with the absorbed lead of 1.364±0.0028 mg.L^{-1} and an absorption value of 99.71%. The water quality showed that parameters of Dissolved Oxygen (DO), salinity, and nitrates from Gresik Regency were not above standard due to heavy metal pollution. S. crassifolium treatment could decrease the nitrite and nitrate values while increasing DO although still below the standard. This study indicated that 10 g alginate of S. crassifolium in 14 days was effective as a bio-absorbent for Pb heavy metal.

Keywords: *Algae, Absorption, Water Pollution, Heavy Metal, Gresik*

Introduction

Gresik is one of Indonesia regency with vast potential in the fisheries sector. The waters area of Gresik Regency is 5,773.80 km^2 (Ilmi and Riniwati, 2018). Its fishery sub-sector has a production value of 287,206,353 ton (Alfian and Akbar, 2020). Another study showed that Gresik Regency covered by 63% total fish pond in East Java and there were 2,489,733 ind.ha^{-1} benthic and 27 ind.ha^{-1} nekton density (Hardini, 2019; Budiman and Damar, 2021). However, the fisheries potential in Gresik is threatened by sub-sector processing industry. Based on industrial data, there were 626 small as well as 706 medium and large industries with various activities, but in 2009 only 22 had Environmental Impact Analysis (EIA) documents and 226 had Environmental Management Plan/Environmental Monitoring Plan documents (Lestari and Budiyanto, 2013).

The processing industry, such as iron and steel, and pulp industry are the two largest water-polluting industries. The relatively fixed ranking for the water pollution and toxin producing industries is more due to the increased total emission activity by the industry, for example, the iron and steel industry reached 62.59% in producing water pollution emissions (Hermawan, 2003). A previous study showed that industrial heavy metal waste in Gresik waters consisted of Hg 0.13 mg.kg^{-1}, Cd 0.64 mg.kg^{-1}, Cu 85.5 mg.kg^{-1}, Pb 4.29 mg.kg^{-1}, and Zn 133 mg.kg^{-1} (Lestari and Budiyanto, 2013). The number of these industries can increase the Pb heavy metal pollution

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*) Corresponding author

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in the fishery, tourism even public health sector. Another study showed that the Pb content near the industrial, residential, and sea areas reached 0.1352, 0.2137, and 0.049 $mg.L^{-1}$ (Purnomo and Muchyiddin, 2008). The lead might accumulate in aquatic organisms, namely the Mozambique tilapia (*Oreochromis mossambicus*) and green mussels (*Perna viridis*) which was commonly consumed by the local community (Awaliyah and Hudha, 2021; Priambodo *et al*., 2021). Lead is the second toxic metal on the earth and affects human health by decrease children's IQ, body impairment, even death (Pain *et al*., 2019; Rahman and Singh, 2019; Usman *et al*., 2020).

Several methods are already applied to decrease heavy metal contents in water such as absorption, deposition, and ion exchange (Renu *et al*., 2017). Among these methods, absorption is the most commonly used because it is simpler and more economical (Ince and Ince, 2017). One of the promising absorbent sources is seaweed due to its availability and cheap price (Utomo *et al*., 2016). Most of the seaweed surface is negatively charged, thus it has a great affinity for metal cations (Fiset *et al*., 2008).

Biosorption is the ability of biological materials, in this case seaweed, to accumulate heavy metals through metabolic media. This biosorption process can occur because of the presence of biological materials called bio absorbents and the presence of solutions containing heavy metals so they are easily bound to the bio absorbents (Pathania, 2016). This research will test the absorption of heavy metal ions by *Sargassum* seaweed (*S. crassifolium*) in the form of wet, dry and alginate. The wet seaweed used in this study were collected from Talango Island, Sumenep, Madura. The seaweed was sun-dried for 2 days to get the dry form while the alginate was obtained by extraction. Lead was chosen due to its highest concentration in Gresik waters and its ability to change organism histopathology (Purnomo and Muchyiddin, 2008; Saad, 2017). The objective of this study was to find the effect of *S. crassifolium* with different form (wet, dry, and alginate) to absorb Pb content in water.

Materials and Methods

The water sample was obtained from Ujung Pangkah sub-district, Gresik Regency (Point 1: 6°85’8.43”S, 112°60’44.27”E, Point 2: 6°84’1.55’’ S, 112°64’21.93’’E, Point 3: 6°87’9.05’’S, 112°63’ 1.55’’S) (Figure 1). Water quality measurements and extraction of *S. crassifolium* (alginate) were carried out at the Surabaya Industrial Research and Standardization Center and the Lamongan Fisheries Service. Samples of *S. crassifolium* were collected from Talango Island, Sumenep Regency, Madura Island (7°08’87.15”S, 114°01’3.12”E).

Heavy metal measurement

Heavy metal was measured using the Atomic Absorption Spectrophotometry (AAS) (Qiu *et al*., 2021). The optimum conditions for the elemental analysis of lead in the AAS was obtained by measuring the absorption at a certain wavelength.

Figure 1. Heavy metal measurement location point on Gresik Regency

Preparation of Sargassum crassifolium biomass

S. crassifolium were cultivated in a photobioreactor by aeration in 24 h lighting conditions (temperature 28-30ºC) in the laboratory. Dry seaweed was obtained by sun-drying for 2 d and 2 kg dry seaweed were weighed, washed, and soaked in 1% HCl for 1 h with a ratio of 1:30 seaweed and water (w/v) (Nurkhanifah and Husni, 2020).

Alginate extraction

Extraction was conducted using Na_2CO_3 solution with varying concentrations of 2%, 4%, 7.5%, 8%, and 10% and the process was carried out twice. Seaweed was extracted for 60 mins, the extract was filtered with a vibrator screen (150 mesh size) and was added with 4% NaOCl solution to the filtrate (Setyoaji *et al*., 2019).

Immobilization of Na-alginate

Alginic acid was formed by adding 10% HCl solution to the filtrate until the pH value was 3 and let stand for one hour. The alginic acid formed was filtered and washed with a vibrator screen and rinsed with water. The alginic acid deposition process used 10% NaOH which was added to the alginic acid gel then stirred until it was homogeneous and reached a neutral pH (6-7). The alginate solution was added to IPA (Isopropyl Alcohol) while stirring until sodium alginate fibers were formed. The fiber was taken and then dried in a dryer which was then milled and sieved into 100 mesh and 80 mesh sodium alginate flour.

Alginate characterization and S. crassifolium biomass

Sodium alginate was analyzed for water content and pH while the structures were identified using FTIR (Fourier Transform Infra-Red).

Treatment of variations in contact time, and absorbent mass

For the experiment on the contact time, a total of 200 mL of water samples containing lead (1.37 mg.L^{-1}) were put in a 250 mL erlenmeyer. Ten gram of wet, dry, and alginate of *S. crassifolium* were put at the lead-containing erlenmeyer then let stand for 14 days. In days 1, 7, and 14, approximately 10 mL water was taken and lead concentration in the solution were measured using AAS. This study used 5 replications for each treatment. The final lead (C_t) concentration was calculated using a calibration curve. The concentration of absorbed lead (C_0) was the difference between C_t and C_0. Meanwhile, the absorption capacity was obtained from the difference between C_t and C_0 and multiplied by the volume of the solution and divided by the mass of the absorbent. Based on (Mulyawan *et al*., 2015) the absorption effectiveness and absorption capacity were calculated based on the following equation:

$$\text{Absorption (effectiveness)} = \frac{Ct}{Co} x 100\%$$

Note: C_t = final concentration of Lead (mg.L^{-1}); C_o = initial concentration of Lead (mg.L^{-1})

For treatment of varied absorbent biomass, 250 mL glass beakers were prepared to contain lead (1.37 mg.L^{-1}), and were added the each treatments of 10, 20, and 30 gr of alginate into each beaker glass. The treatment was conducted for 7 days and at the end the lead concentration was measured using AAS at a 283.3 nm wavelength.

Data analysis

To determine different effects of those three variables (wet, dry, and alginate form of *S. crassifolium*) based on contact time and absorbent methods, Anova Test ($p<0.05$) was applied. To determine the effect of the treatments, the result will be followed by Duncan's Multiple Range Test at 95% confidence.

Result and Discussion

Pb content in Ujung Pangkah, Gresik Regency waters was 1.371 mg.L^{-1}. See Table 1. The value exceeded the standards of biota life in Indonesia (<0.05 mg.L^{-1}) and EQS/ Environmental Quality Standards (<0.15 mg.L^{-1}). It is indicated that the waters in Gresik Regency in high level of lead pollution, which can be associated with the poor and inadequate waste water treatment of nearby industries. Gresik had many industrial factories (626 small industries and 706 medium and large industries) and only 22 had EIA (Environmental Impact Analysis) certificates and 226 had Environmental Management Plan/ Environmental Monitoring Plan documents in 2009 (Lestari and Budiyanto, 2013).

This condition confirms with previous research statements that besides factories, household and agricultural wastes affected the water quality of Gresik Regency (Aminin *et al*., 2020). The number of these industries can caused increasing the heavy metal (lead) pollution to marine fishery sector (Ansari *et al*., 2004). This waste has affected the surrounding environment, including biota and even humans.

Several biotas in Gresik that has been exposed to Pb are Mozambique tilapia (*Oreochromis mossambicus*), mangrove (*Avicennia marina*), and green mussels (*Perna viridis*) (Maharani *et al*., 2019; Rayyan *et al*., 2019). The declining water quality also affects shrimp farmers in Ujung Pangkah to lose their job due to mortality of their cultivation organism (Iranawati *et al*., 2020). Humans who eat these biotas also experience health risks. Lead metabolism in the body will first be absorbed by the inner lungs (alveoli). Usually, it stays 24 hours and then goes to the bloodstream. After lead is absorbed in the blood plasma, it will immediately be transferred to blood cells, especially in red blood cells (95-98%).

Pb is easily absorbed wherever high levels of calcium are found. Therefore, the highest long-term concentrations were found in bone, particularly dense cortical bone. Also, the highest Pb concentration in the body is easily found in organs and tissues that have the highest mitochondrial activity such as the renal tubule, choroid plexus, and cerebellum of the central nervous system. After the lead is distributed evenly, some will be excreted in the form of urine and feces. Approximately 70% of Pb intake will be excreted through urine, partly in feces which is the amount of endogenous lead Pb that is not absorbed from saliva, bile and to minimize other digestive secretions, as well as other incomplete absorption residues by the body. As a result, Pb inhibits several enzymes *en route*, especially porphobilinogen synthetase and heme synthetase (Syakbanah, 2018).

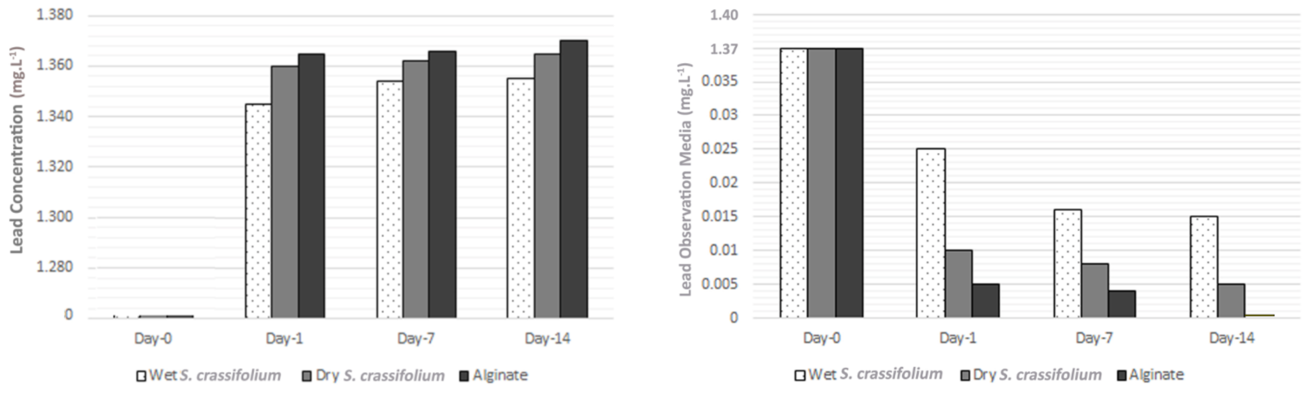
There was a time effect on the absorption process of lead metal ions (Figure 2.). Based on these data, the metal ion absorption process in the three treatments was increasing and Pb content in the water sample was decreasing in 14 days. It is indicated that *S. crassifolium* can absorb Pb content in waters in the form of wet, dry, and alginate. The research and application of *Sargassum* to remove Pb from water has been reported (Cardoso *et al*., 2016; Hannachi and Hafidh, 2020). This biosorption process can occur because of the presence of biological materials called bio absorbents and the presence of solutions containing heavy metals so that they are easily bound to the bio absorbents (Pathania, 2016).

There was a significant difference ($p<0.05$) of lead content among the treatments (Table 2.). The highest absorption level showed at alginate form in 14 days with the value of 1.370±0,0034 $mg.L^{-1}$. Alginate of *S. crassifolium* had a high absorbent power compared to wet and dry *S. crassifolium*. Previous study also stated that the contact time was a significant factor in the absorption process of the heavy metal of ion lead (Plazinski, 2012). The increasing time for absorption process provides an opportunity for the absorbent to adapt to the environment and remove the heavy metal (Wulandari *et al*., 2014). Although the difference between treatments was low (0,004-0,015 $mg.L^{-1}$), the results showed a significant value between treatments.

Table 1. Levels of lead heavy metals in the waters of Gresik Regency

No	Point Location	Lead (Pb) ($mg.L^{-1}$)	Standard in Indonesia*	EQS**
1	Ujung Pangkah 1	1.37	<0.05	<0.15
2	Ujung Pangkah 2	1.37	<0.05	<0.15
3	Ujung Pangkah 3	1.37	<0.05	<0.15

Note : * = Hariyanti *et al*., 2021; ** = Gao *et al*., 2021



(a) (b)

Figure 2. (a) Concentration of lead absorbed at different contact times by *S. crassifolium,* (b) Concentration of lead in water (media)

It suggested that heavy metals could have a harmful effect even if only in small amounts (Agustina and Tjahjaningsih, 2021).

Research related to the effect of Pb on the brain on organisms in postnatal development reported that a difference of <1 mg.L^{-1} caused a significant difference and affect the work of enzymes (Dearth *et al*., 2002). Aquatic organisms only require fewer heavy metals than 0.05 mg.L^{-1} (Hariyanti *et al*., 2021). If the absorbed heavy metal exceeds the recommended limit, it will affect the body.

The percentage of Pb absorption based on contact time showed significant difference ($p<0.05$). See Table 3. Duncan's Multiple RangeTest results showed that alginate on day 14 with a value of 100% was the best result. These results indicated that *S. crassifolium* alginate had better Pb metal removal ability than some previously reported materials, such as tea waste (98%), *Peganumharmala* seeds (96-97%), and rice husk (90%) (Gaur *et al*., 2018). This absorption percentage is critical since it evaluate the absorbent capability to provide safe water for aquatic organisms and prevent death (Oboh and Aluyor, 2008). Alginate is a polymer consisting of mannuronic and guluronic. Alginate has been widely used by various industries as a thickening agent, balance regulator, emulsifier, and oil-resistant film-forming agent. In addition, alginate is also known to have a high affinity for heavy metals and radioactive elements, so these compounds can help in cleaning up heavy metal and radioactive pollution in the food consumed (Goh *et al*., 2012). Alginate of seaweed is already used for Pb removal in many cases (Aden *et al*., 2019; Varaprasad *et al*., 2020). The absorption ability of macroalgal biomass varies taxonomically, some of the acids responsible for metal absorption are as follows: sulfated polysaccharide esters (fucoidans, carrageenan, and Galatia and xylan) and polyuronides (galacturonic, glucuronic, guluronic, and mannuronic acids) (Hammud *et al*., 2014).

There was a decrease in the absorption value of the lead-heavy metal ions with *S. crassifolium* treatments (Figure 3.). The use of alginate from seaweed to overcome lead pollution has been conducted in various countries such as Brazil (using *S. filipendula*) and Chile (using *Laminaria digitata*) (Cardoso *et al*., 2016; Varaprasad *et al.,* 2020). In Indonesia, research regarding with alginate of *S. crassifolium* to reduce lead content in water has never been reported. But previous research has stated that *S. crassifolium* in dry form has been used to absorb heavy metals in Indonesia (Putri and Syafiqa, 2019). This study is the first reported research on the use of alginate of *S. crassifolium* to absorb lead. Alginate has advantages due to its solubility and ability to bind to water depends on the number of carbohydrate ions, molecular weight, and pH. The ability to bind water increases if the number of carbohydrate ions increases, while at pH below 3 there is precipitation. Alginate has the main properties to dissolve in water and increase the viscosity of the solution, the ability to form gels and films (Lee and Mooney, 2012). This biosorption process is carried out by modifying the biomass by means of immobilization. The immobilized biomass on the porous support will further expand the surface and larger pore volume and have high particle strength, porosity, chemical resistance, and surface area (Zhang *et al*., 2013).

Table 2. Duncan's multiple range test concentration based on contact time

Bio absorbent	Absorbed Lead Concentration (mg.L^{-1}) ± SD		
	Day-1	Day-7	Day-14
Wet *S. crassifolium*	1.345^{a} ± 0,0064	1.354^{a} ± 0,0019	1.355^{b} ± 0,0019
Dry *S. crassifolium*	1.360^{ab} ± 0,0015	1.362^{a} ± 0,0020	1.365^{b} ± 0,0027
Alginate	1.365^{b} ± 0,0021	1.366^{b} ± 0,0020	1.370^{a} ± 0,0034

Note: Letter notation shows the comparison between treatments, there is a significant difference ($p<0.05$) in Duncan's multiple range test

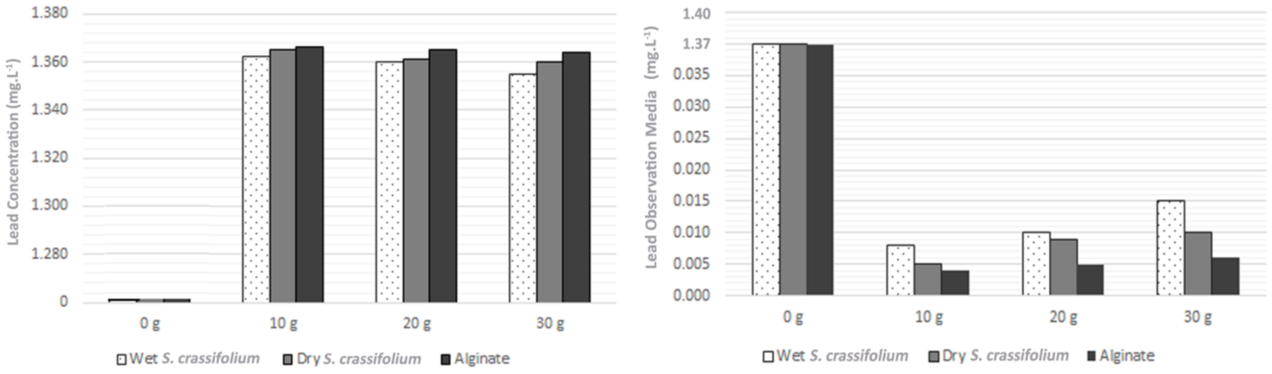
Table 3. Percentage of Pb absorption based on contact time

Bio absorbent	Absorption (%)		
	Day-1	Day-7	Day-14
Wet *S. crassifolium*	98.18^{a}	98.83^{a}	98.91^{b}
Dry *S. crassifolium*	99.27^{ab}	99.42^{b}	99.64^{b}
Alginate	99.64^{b}	99.71^{b}	100^{a}

Note: Letter notation shows the comparison between treatments there is a significant difference ($p<0.05$) in Duncan's multiple range test

The data in Table 4 showed a significant difference ($p<0.05$) on concentration based on absorbent mass among the treatments. The Duncan's Multiple Range Test revealed that the highest absorption level showed there is no significant difference among the 10, 20, and 30 gr alginate. Thus, 10 gr alginate were recommended because it did not need extra biomass, least cost and effort to be prepared. It happened because alginate has low mechanical strength and stability that affect absorption though the biomass increases (Gao *et al*., 2020). Similar result was also reported in previous study that increasing biomass induce overlapping absorption as a result of overcrowding particles (Gaur *et al*., 2018). Studies regarding physical and chemical modifications to overcome these cases have been conducted (Qu *et al*., 2020).

In all treatments, 10 gr biomass showed the highest value of absorbed lead concentration. The increase in the mass of the absorbent means that the surface area was greater, thus caused the removal efficiency value is also increase. The decrease in lead heavy metal solutes was due to saturation of the pores of the bio absorbent surface (Mulyawan *et al*., 2015). However, the increase in removal efficiency with a greater mass will reduce the absorption capacity. This decrease in absorption capacity will result in desorption. Desorption is a condition in which the absorbent is saturated or near saturation, so the absorbate absorbed then will be released from the absorbent and return to become an impurity in the sample, thereby reducing removal efficiency (Nurhasni *et al*., 2012). The absorption process is stated to stop because based on the percentage of absorbed Pb it has approached equilibrium because the number of absorbate molecules that bind to the absorbent is getting smaller. The amount of absorbent affects the absorption process where the increasing weight causes the absorbent to reach its saturation point if the surface has been filled with absorbate (Ningsih *et al*., 2016).



(a) (b)

Figure 3. (a) Concentration of lead absorbed at different biomass by *S. crassifolium*, (b) Lead Concentration in water (medium)

Table 4. Duncan's multiple range test concentration based on absorbent mass

Bio absorbent	Absorbed Lead Concentration (mg.L^{-1}) ± SD		
	10 g	20 g	30 g
Wet *S. crassifolium*	1.362^{a} ± 0,0090	1.360^{a} ± 0,0025	1.355^{a} ± 0,0014
Dry *S. crassifolium*	1.365^{ab} ± 0,0010	1.361^{ab} ± 0,0026	1.360^{ab} ± 0,0014
Alginate	1.366^{b} ± 0,0030	1.365^{b} ± 0,0020	1.364^{b} ± 0,0028

Note: Letter notation shows the comparison between treatments there is a significant difference ($p<0.05$) in Duncan's multiple range test

Table 5. Percentage of Pb absorption based on absorbent mass

Bio absorbent	Absorption (%)		
	10 g	20 g	30 g
Wet *S. crassifolium*	99.41^{a}	99.27^{a}	98.91^{a}
Dry *S. crassifolium*	99.64^{ab}	99.34^{ab}	99.27^{ab}
Alginate	99.71^{b}	99.64^{b}	99.56^{b}

Note: Letter notation shows the comparison between treatments there is a significant difference ($p<0.05$) in Duncan's multiple range test

Table 6. The average value of the treated water quality

Parameter	Unit	Initial	Wet *S. crassifolium*	Dry *S. crassifolium*	Alginate *S. crassifolium*	Standard*
pH		8	7.86	7.45	6.04	7-8.5
Temperature	^{0}C	29	29	29.5	30.1	28-30
DO	mg.L^{-1}	2	3.23	3.45	3.33	>5
Salinity	0/00	29	29	29	29	33-34
Nitrate	mg.L^{-1}	1	0.7	0.8	0.5	0.008
Nitrite	mg.L^{-1}	0.12	0.11	0.13	0.09	5

* Patty *et al.* (2019)

There was a significant difference ($p<0.05$) Pb absorption based on absorbent mass between the treatments Table 5. According to Duncan's Multiple Range Test, there was no significant difference lead absorption among 10, 20, and 30 g alginate of *S. crassifolium*. It is indicated that alginate is more effectively used as a bio absorbent for the heavy metal ion Pb. This is in accordance with the previous work that stated the carboxylic acid in the form of OH in the carbonyl group in the alginate could remove heavy metal ions in the network and also in the waters by binding the heavy metal ions to form complex compounds (Kama *et al*., 2020).

The data showed that water qualities of DO (Dissolved Oxygen), salinity, and nitrates from Gresik Regency were below the standard (Table 6.). This was due to heavy metal pollution that entered the water. Previous studies stated that the entry of heavy metals into waters such as lead played a role in lowering DO (Sekabira *et al*., 2010). This lack of oxygen was also affected by nitrates. High nitrate is caused by pollution in Gresik Regency lead to high oxygen demand and reduced DO (Osman and Kloas, 2010). Salinity was also affected by heavy metals. Keniston (2015) showed that salinity was inversely related to the amount of heavy metal exposure.

The data also indicated that the treatment of *S. crassifolium* could decrease the water parameter of nitrite, nitrate, and increase DO although they did not meet the standard. The pH value of the alginate was lower when compared to the two treatments. *S. crassifolium* could decrease nitrate and nitrite although they did not meet standard for biota in the water. Interestingly the pH was not above the standard (6.04). This was due to the change of carboxylate groups to protonate and form hydrogen bonds due to alginate content in water (Lee and Money, 2012). García-Rosales *et al*. (2012) stated that Pb ions could be absorbed optimally at pH 1-6 so the alginate treatment could decrease heavy metal better than others. This was because the Pb ion was acidic. The temperature in the alginate also supported the absorption power because the higher the temperature value, the higher the absorption of heavy metals. This was in accordance with Yadava *et al*. (1991) that temperature greatly affected the absorption of lead ions because the higher the temperature value caused collisions between molecules so that the molecules would be unstable.

Conclusion

The lead concentration in Gresik Regency exceeds the standard. The highest decrease of lead content is found in alginate of *S. crassifolium* absorbent treatment with 14 days of contact time and biomass weight of 10 gr. This study suggested that *S. crassifolium* alginate can be used as a bio absorbent to overcome heavy metal lead pollution in Gresik Regency.

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