Heavy Metal (Pb) and Its Bioaccumulation in Red Algae (*Gracilaria* sp.) At Kupang Village, Jabon Sub-District, Sidoarjo District

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Abstract

Seaweeds have an inherent capacity to absorb heavy metals from marine water. This intrinsic ability allows these organisms to accumulate much amount of heavy metals over time. This study conducted at Kupang Village, Jabon Sub-District, Sidoarjo District, where almost 40% of the area consists of *Gracilaria* sp. ponds cultured. The purpose of this study was to analyze the Pb heavy metals concentration in water culture and its concentration in holdfast and thallus of *Gracilaria* sp. In this research, determination of samples in each pond was taken on day 0 (before planting), 20 days (Initial Production) and on day 40th (Post/Harvest). The aquatic parameters include salinity, temperature, acidity (pH), and dissolved oxygen (DO). Quantitative determination of heavy metals on sample using Atomic Absorption Spectrophotometry (AAS). Determinations of heavy metal accumulation in organism using Bioconcentratin factor (BCF) and Translocation factor was used to calculate the Pb heavy metal translocation process from the base to the tip of *Gracilaria* sp. The results of the study showed the highest Pb concentration found in the second sampling age 20 days, in pond 1 with 7.61 ± 0.18 $mg.kg^{-1}$, and pond 2 was 5.35 ± 0.09 $mg.kg^{-1}$. This concentration has not exceeded the threshold value that might have an effect if more than 8.6 $mg.kg^{-1}$. The highest Pb level at the holdfast of *Gracilaria* sp. found at age 0 days before planting, which is 3.38 ± 0.23 $mg.kg^{-1}$ and decreases to post-harvest (age of 40 days) which is 0.84 ± 0.00 $mg.kg^{-1}$. The Transaction Factor (TF) of Pb heavy metal value from holdfast to thallus is 1,015 thus *Gracilaria* sp. absorbs heavy metals in high concentrations at the beginning of planting and is able to release it again before harvest time.

Keywords: Bioaccumulation, *Gracillaria* sp., Heavy Metal, Histological, Red Algae.

INTRODUCTION

Red seaweed *Gracilaria changi* is a good potential source of β-carotene due to the established high content of 5.2 $mg.100g^{-1}$, which is in comparison with 6.8 $mg.100g^{-1}$ in carrots [1]. Meanwhile, *Kappaphycus alvarezii* is commercially important of red algae as a phycocolloid that is extensively applied as a thickening and stabilizing agent in food, pharmaceutical, and cosmetic industries and needed for its cell wall polysaccharide. Also, it has been used in health beverages and anticancer nutraceutical because of its antioxidant content and other nutritive compounds [2].

Heavy metals are significant environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons [3,4]. The most commonly found heavy metals in water include arsenic, cadmium, chromium, copper, lead, nickel, and zinc, all of which cause risks for human health if consumed usually through food [5].

Kupang Village is one of the villages located in Jabon Sub-District, Sidoarjo District, where almost 40% of the area consists of ponds. One of the leading cultivation in the village is *Gracilaria* sp. seaweed. The presence of airflow from the Porong River is known to contain heavy metals into the aquaculture environment of *Gracilaria* sp. The previous study stated that the increased lead (Pb) over the threshold in the Porong River is quite dangerous for biota [6]. Also, the results of heavy metal measurements carried out at the Porong river estuary proved that heavy metal lead (Pb) is at the threshold set by the Government with a Pb content of 0.0648 ppm [7].

Seaweeds have an inherent capacity to absorb heavy metals from marine water. This intrinsic ability allows these organisms to accumulate much amount of heavy metals over time. Previous investigations had observed the accumulation of the selected heavy metals in the different species of seaweeds. The study showed that five species of seaweeds have accumulated seven types of heavy metals (Cd, Cu, Mn, Ni, Pb, Zi, and Hg) at varying concentrations [8].

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The absorption (accumulation) process also influenced by the form of the thallus, e.g. at the base and branching of *Gracilaria* sp. Naturally, the thallus that located at the base part which attached itself to the substrate has different absorption process from the tip thallus *Gracilaria* sp. [9]. The purpose of this study was to analyze the accumulator potential of *Gracilaria* sp through determinations of the concentration and effect of Pb heavy metals on the holdfast and thallus of the talus seaweed tissue.

MATERIAL AND METHOD

This research was conducted in July-September 2018, where sampling was carried out in Kupang Village, JabonSub-district, Sidoarjo. Sampling of *Gracilaria* sp. seaweed conducted in 2 (two) ponds. From each pond, the best quality seaweed was taken as much as 1 kg. Determination of samples in each pond was taken on day 0 (before planting), 20 days (Initial Production) and on day 40th (Post/Harvest).

Aquatic Parameter

Aquatic parameters observed directly on *Gracilaria* sp. ponds. The aquatic parameters include salinity, temperature, acidity (pH), and dissolved oxygen (DO) [10].

Atomic Absorption Spectrophotometry (AAS)

Atomic Absorption Spectrophotometry (AAS) is an analytical technique that measures the concentration of elements contained in the *Gracilaria* sp sample. It is the basis for the quantitative determination of metals by using Atomic Absorption Spectrophotometry [11,12].

Data Analysis

Data obtained from the results of the study were analyzed descriptively quantitatively. AAS was used to determine the content of heavy metal lead (Pb) and Bioconcentratin factor (BCF) to calculate the value of heavy metal accumulation in organisms with the formula:

$$BCF = \frac{C_{org}}{C_{water}}$$

Description:
BCF = Bioconcentratin factor,
$C_{organisme}$ = metal content in organisms (mg.kg^{-1})
C_{water} = metal content in water (mg.kg^{-1}).
Category of BCF values [11]:
> 1000 = high accumulative properties category
100-1000 = medium accumulative properties
< 100 = low accumulative properties

Translocation factor was used to calculate the Pb heavy metal translocation process from the base to the tip of *Gracilaria* sp. [12], by the formula:

$$TF = \frac{BCF_{thallus}}{BCF_{holdfast}}$$

Categories:
TF > 1 = Phytoextraction mechanism
TF < 1 = Phytostabilization mechanism

RESULT AND DISCUSSION

Aquatic parameter

The parameters of the water sample which measurements are made directly in the field can be seen in Table 1. The results of salinity measurements at the research station exceeded the SNI 7904: 2013 threshold of 15-30‰ [13]. One of the factors that cause salinity levels increases because the sampling conducted in the dry season (July-August), so that the salt content increases due to evaporation. However, *Gracillaria* sp. has the ability to tolerate high salinity levels [14].

Table 1. Water Quality in *Gracilaria* sp. Sampling Water

Parameter	***Gracilaria* sp. production age**					
	Pond 1 (Days)			**Pond 2 (Days)**		
	0	**20**	**40**	**0**	**20**	**40**
Salinity (‰)	33	35	41	33	36	35
Temperature (°C)	27	25	25	27	24	25
pH	7.5	6.8	6.9	7.5	7.3	7.0
DO (mg.L^{-1})	5	8	8	8	11	5

The results of temperature measurements on pond 1 and pond 2 of *Gracilaria* sp. in Kupang Village, is still in the range of SNI 7904; 2013 [13], i.e. 20-28°C; which is still considered good for the growth of *Gracilaria* sp. Thus, it can improve the process of nutrients absorption and accelerate the growth rate of seaweed.

Measurement of the degree of acidity (pH) in the *Gracilaria* sp. pond range for Pond 1 is 6.8-7.5 and in Pond 2 is 7.0-7.5. The pH range on Pond 1 and Pond 2 is still in the range of SNI 7578: 2010 [15], namely 6.8-8.2. Waters with a pH value = 7 are neutral, pH < 7 is said to be an acidic water condition [16]. Decreasing pH in Pond 1 which less than 7 and said to be acidic will cause greater heavy metal toxicity. The decrease in pH in the waters can also cause greater levels of pollutant bioaccumulation in organisms [17].

Dissolved oxygen content in the study location both on Pond 1 and Pond 2 has not exceeded the threshold determined by SNI 7578: 2010 [15] which is > 3.0 mg.L^{-1}. The DO content which is always above 3.0 mg.L^{-1}, can be due to research conducted in the morning before noon

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where photosynthesis takes place at that time. It causes the addition of oxygen through photosynthetic processes and the exchange of gas between water and air which causes relative dissolved oxygen levels higher [18,19].

Pb Metal Content

In Water

The Pb concentration analysis on *Gracilaria* sp. water sampling using the AAS method showed in Figure 1. In the first, second, and third water samples, the highest concentration of Pb found in water sampling before planting on Pond 1 and Pond 2, and then there was a decrease in the second and third sampling. Pb heavy metals in the waters were found in dissolved and suspended form. Lead solubility in water was quite low, so the levels were relatively small [20]. Furthermore, there was a decrease in ponds aged 40 days, which were below the threshold value of 0.09 mg.L^{-1}, allegedly exchanging lead into sediments through binding to organic matter [21].

In the Sediments

The level of heavy metals Pb in the sediment of ponds with *Gracilaria* sp. using AAS, seen in Figure 2. The highest Pb content found in the second sampling, age 20 days in pond 1 was 7.61 ± 0.18 mg.kg^{-1} and in the second pond was 5.35 ± 0.09 mg.kg^{-1}. This concentration may be caused by human activity around this area. Various sources of heavy metals include soil erosion, natural weathering of the earth's crust, mining, industrial effluents, urban runoff, sewage discharge, insect or disease control agents applied to crops, and many others [22]. This concentration has not exceeded the threshold value that might have an effect of more than 8.6 mg.kg^{-1} [23]. It means that the concentration of Pb is not too dangerous for living things.

In Holdfast of Gracilaria sp.

Measurement of Pb content in the holdfast of *Gracilaria* sp. in Kupang Village, using AAS method, can be seen in Figure 3. The highest Pb level in the holdfast of *Gracilaria* sp. found at age 0 days before planting, 3.38 ± 0.23 mg.L^{-1}, and then it decreased to post-harvest age of 40 days which is 0.84 ± 0.00 mg.L^{-1}.

Gracilaria sp. before planting is the seedlings that originated from the same aquaculture pond in the form of cuttings selected from fresh plants [24], thus experiencing stress [25] and experiencing heavy Pb heavy metal absorption. Pb Content in Pond 1 at 20 days decreased more than Pond 2. It is due to in the times of adaptation and at the age of 20 days, the Pb absorption process in *Gracilaria* sp. was very strong. The nutrients for breeding and Pb will also be absorbed.

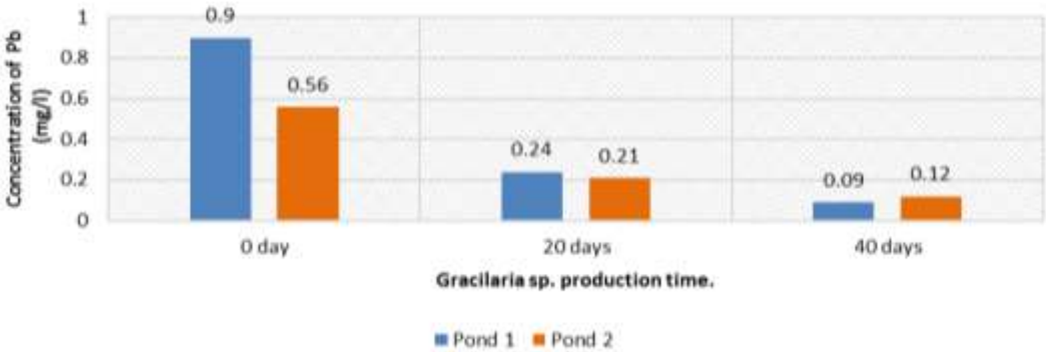

Figure 1. Pb Concentration in Water

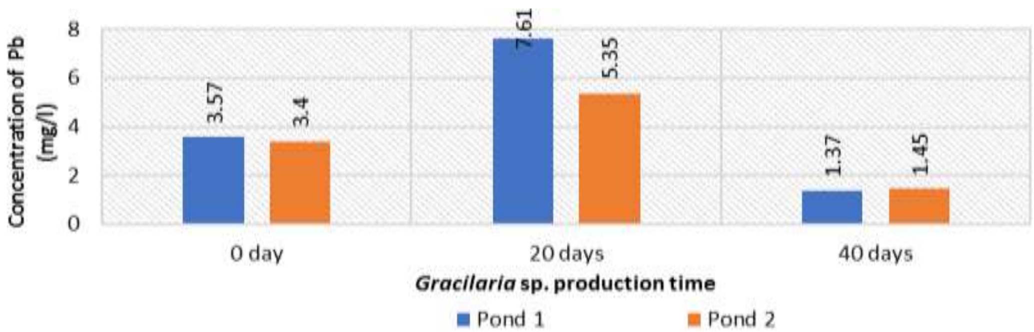

Figure 2. Pb Concentration in Sediment

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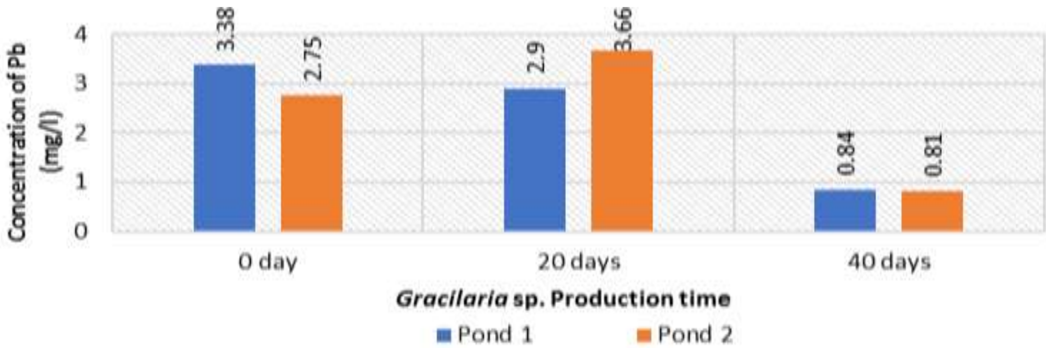

Figure 3. Pb Concentration in holdfast of *Gracilaria* sp.

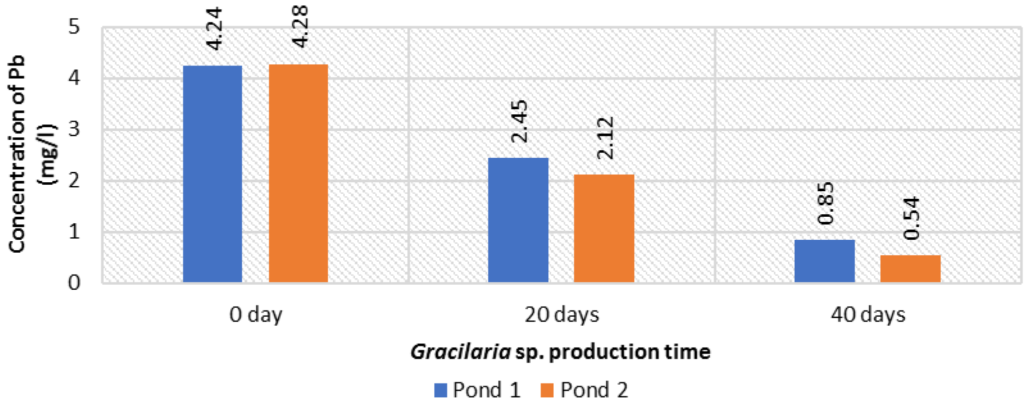

Figure 4. Pb Concentration in the Young Thallus of *Gracilaria* sp.

The high adaptation ability of *Gracilaria* sp. to change the water quality makes it able to survive in the stress of heavy metals without disrupting the growth rate. The growth rate of *Gracilaria* sp. at the end of the cell meristem is higher than the base. It is because, at the end of the cell, the branching is large, small and still young, while at the base consists of cells that are old with a larger but smaller number of branches [26].

The results of the analysis using AAS at the young thallus of *Gracilaria* sp. showed high Pb levels at the first sampling at the age of 0 days for 4.24 $mg.L^{-1}$. It then decreased at the age of 20 days to 2.12 $mg.L^{-1}$ and age 40 days to 0.54 $mg.L^{-1}$ (Fig. 4).

The process of Pb absorption by *Gracilaria* sp. influenced by the shape of the thallus. The shape of the *Gracilaria* thallus is cylindrical and forms clumps with irregular branching types. Otherwise, it does not shape like that at the base, because it has old tissue properties, thus it does not have the activity to multiply itself [27]. Material that has smaller size in diameter has a higher absorption rate than a larger diameter adsorbent [28].

From the results of observations and measurements of Pb heavy metal concentrations at the holdfast and thallus of *Gracilaria* sp., it can be used as a bioaccumulator; as well as histology at the holdfast and thallus of *Gracilaria* sp. can also be used as an accumulator. Parts of seaweed in general consist of the holdfast, which is the basic part of seaweed with functions to stick to the substrate, and thallus which is a form of seaweed growth that resembles branching [29].

Bioconcentration factor (*BCF*)

In Water and Sediment

The bioconcentration of Pb heavy metals in water and sediment are shown in Table 2. The results of the sediment BCF calculation in the water with *Gracilaria* sp. treatment age 0 days, 20 days and 40 days included in the category of low accumulation group.

It was also supported by using the t- test analysis, where significance value (t-tailed) is 0.000 < 0.05. It indicates a significant difference at the 95% level of confidence that there is an influence between the concentration of Pb in water and sediment on the growth of *Gracilaria* sp. [30].

Table 2. Concentration of Pb in Water and Sediment

Sample	Heavy metal concentration (Pb) and BCF value		
	C Water	*C Sediment*	BCF Value
$P1_0$	0.90	3.57	3.967
$P2_0$	0.56	3.40	6.071
$P1_{20}$	0.24	7.61	31.708
$P2_{20}$	0.21	5.35	25.476
$P1_{40}$	0.09	1.37	15.222
$P2_{40}$	0.12	1.45	12.083

Description:
$P1_0$ = Pond 1 first sampling (age 0 days)
$P2_0$ = Pond 2 first sampling (age 0 days)
$P1_{20}$ = Pond 1 second sampling (aged 20 days)
$P2_{20}$ = Pond 2 second sampling (aged 20 days)
$P1_{40}$ = Pond 1 third sampling (aged 40 days)
$P2_{40}$ = Pond 2 third sampling (aged 40 days)

Low concentration of Pb heavy metal in the water because of Pb is mostly found in the sediment, thus indicating that most of the Pb compounds in Pond 1 and Pond 2 are in the form of particles or deposits. Anthropogenic materials such as Pb heavy metals carried by the air from factories and population activities will settle on sediments [31].

In Water and Holdfast of Gracilaria sp.

Bioconcentration factor of Pb on the seaweed showed in Table 3. The first sampling has a low Pb BCF because this sampling was carried out where *Gracilaria* sp. has not spread on the pond. Accumulatively, the Pb BCF value is still low, supported by using the t- test, where significance value (t-tailed) is 0.000 < 0.05. The results prove that the Pb heavy metal concentration varies significantly with the absorption of Pb heavy metal in the base of water [31].

Table 3. Heavy Metal Concentration (Pb) in Water and Holdfast of *Gracilaria* sp.

Sample	Pb concentration and BCF value		
	Water	Holdfast	BCF Value
$P1_0$	0.90	3.38	3.756
$P2_0$	0.56	2.75	4.911
$P1_{20}$	0.24	2.90	12.083
$P2_{20}$	0.21	3.66	17.429
$P1_{40}$	0.09	0.84	9.333
$P2_{40}$	0.12	0.81	6.750

Description:
$P1_0$ = Pond 1 first sampling (age 0 days)
$P2_0$ = Pond 2 first sampling (age 0 days)
$P1_{20}$ = Pond 1 second sampling (aged 20 days)
$P2_{20}$ = Pond 2 second sampling (aged 20 days)
$P1_{40}$ = Pond 1 third sampling (aged 40 days)
$P2_{40}$ = Pond 2 third sampling (aged 40 days)

In Water and Young Thallus of Gracilaria sp.

Pb BCF value at the young thallus of *Gracilaria* sp. showed in Table 4. Pb BCF value in water and the young thallus of *Gracilaria* sp. ranges from 4.5-10.208 (Table 4). By using the t-test, where significance value (t-tailed) is 0.000 < 0.05, so the hypothesis is proven that there is a difference absorption process in the tip of *Gracilaria* sp. for Pb heavy metals. The age of seaweed also greatly affects the process of Pb heavy metals sequestration in *Gracilaria* sp. [32].

Table 4. Heavy Metal Concentration (Pb) in Water and Young Thallus of *Gracilaria* sp.

Sample	Pb concentration and BCF value		
	Water	Young Thallus	BCF value
$P1_0$	0.90	4.24	4.711
$P2_0$	0.56	4.28	7.643
$P1_{20}$	0.24	2.45	10.208
$P2_{20}$	0.21	2.12	10.095
$P1_{40}$	0.09	0.85	9.444
$P2_{40}$	0.12	0.54	4.500

Description:
$P1_0$ = Pond 1 first sampling (age 0 days)
$P2_0$ = Pond 2 first sampling (age 0 days)
$P1_{20}$ = Pond 1 second sampling (aged 20 days)
$P2_{20}$ = Pond 2 second sampling (aged 20 days)
$P1_{40}$ = Pond 1 third sampling (aged 40 days)
$P2_{40}$ = Pond 2 third sampling (aged 40 days)

The results of bioconcentration factors indicate that *Gracilaria* sp. both at the holdfast and thallus can accumulate Pb heavy metals in the waters. A portion of heavy metals absorbed in water and accumulates in the form of particles and then settles on the surface of the sediment [33]. Metals will be bioaccumulated in living organisms or the body of aquatic biota. The amount of metal that accumulates will continue to increase [34].

Translocation Factors (*TF*)

TF analysis is used to calculate the Pb heavy metal translocation process from the base (holdfast) to the tip (young thallus) of *Gracilaria* sp. The value from the calculation of the Transaction Factor (TF), can be seen in Table 5 below.

TF value of Pb from holdfast to thallus is 1,015 so that *Gracilaria* sp. can be regarded as a high-value bioaccumulator. It was also checked with analysis to verify the existence of significant by using the t- test, where significance value (t-tailed) is 0.000 < 0.05. There are several factors that affect the difference in growth in holdfast and thallus *Gracilaria* sp. one of them is existance of heavy metal [33]. The concentration of Pb heavy metals contained in the holdfast and thallus of seaweed *Gracilaria* sp. and the absorption process influences the growth of

Gracilaria sp. Value of TF > 1 shows that plants are enriched with metals as Pb heavy metal accumulators [35].

Tabel 5. TF Concentration on Holdfast to the Young Thallus of *Gracilaria* sp.

Sample	Pb concentration and TF value		
	Young Thallus	Holdfast	TF Value
$P1_0$	4.24	3.38	1.000
$P2_0$	4.28	2.75	2.000
$P1_{20}$	2.45	2.90	0.845
$P2_{20}$	2.12	3.66	0.579
$P1_{40}$	0.85	0.84	1.000
$P2_{40}$	0.54	0.81	0.667
Average			**1.015**

$P1_0$ = Pond 1 first sampling (age 0 days)
$P2_0$ = Pond 2 first sampling (age 0 days)
$P1_{20}$ = Pond 1 second sampling (aged 20 days)
$P2_{20}$ = Pond 2 second sampling (aged 20 days)
$P1_{40}$ = Pond 1 third sampling (aged 40 days)
$P2_{40}$ = Pond 2 third sampling (aged 40 days)

According to Bioconcentration factor (BCF) and Translocation Factors (TF), most of the investigations on microalgae and macrophytic algae were considered as valuable indicators because of their accumulation capacity [34]. Trace elements like manganese, copper, lead, and zinc are present in very small quantities and are considered as the essential micro-nutrients for proper growth of the plants. It is well known that elements such as Cu, Mo, Ni, Cl, and Zn are essential for plant growth in low concentrations [36]. Nevertheless, beyond certain threshold concentrations, these same elements become toxic for most plant species [37,38].

Several plant species are capable of tolerating high concentrations of heavy metals and thus opened new possibilities to use these plants to remediate contaminated soils (phytoreme-diation). Many studies have been conducted to determine the toxic levels of heavy metals for certain plants, especially those metals considered as public health threats [36,39]. At the low concentrations, some of the heavy metals excite some biological processes, but at threshold concentration these become toxic. Being non-biodegradable, these metals accumulate at various trophic levels through the food chain and can cause human health problems [40].

In humans, these metals hoard in living tissues and thus multiply the danger. Some metals cause physical distress while others may cause life-threatening illness, damage to the vital body system, or cause other damages. Thus, it is very essential to control the emission of heavy metals into the environment. Seaweeds are excellent agents of filtering the metals like zinc, cadmium, copper, nickel and iron and some potential carcinogens from seawater.

CONCLUSION

Highest Pb content was found in the second sampling, age 20 days, in Pond 1 for 7.61 ± 0.18 $mg.kg^{-1}$ and the Pond 2 for 5.35 ± 0.09 $mg.kg^{-1}$. This concentration has not exceeded the threshold value that might have an effect of more than 8.6 $mg.kg^{-1}$. Pb level analysis at the holdfast and thallus of *Gracilaria* sp. found the highest Pb content at age 0 days before planting, which is 3.38 ± 0.23 $mg.kg^{-1}$and decreases to post-harvest age of 40 days which is 0.84 ± 0.00 $mg.kg^{-1}$. Transaction Factor (TF) of heavy metal Pb value from holdfast to thallus is 1.015. so *Gracilaria* sp. absorbs heavy metals in high concentrations at the beginning of planting and is able to release it again before harvest time.

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