

THE EFFECT OF AMMONIUM CONCENTRATION ADDITION TO *Gracilaria* sp. ON THE ABSORPTION OF MERCURY

Yudi N Ihsan^{1*}, Luthfiyah Azizah², Kalysta Fellatami³, Tri Dewi K Pribadi⁴

¹Marine Department, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km. 21, Jatinangor, West Java, Indonesia.

²Department of Hydrobiology, University of Debrecen, Egyetem tér 1, 4032, Hungary

³College of Fisheries, Ocean University of China, Qingdao, China

⁴Biology Department, Faculty of Mathematics and Natural Science, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km. 21, Jatinangor, West Java, Indonesia.

*Corresponding author: yudi.ihsan@unpad.ac.id

Submitted: 24 January 2022 / Revised: 24 February 2022 / Accepted: 09 March 2022

<http://doi.org/10.21107/jk.v15i1.13447>

ABSTRACT

The research was conducted from March to June 2015 in the laboratory of Marine Science of the faculty of FPIK UNPAD. The sampling of *Gracilaria* sp. was taken from a seaweed farming in Kalianda, Bandar Lampung Province. The aim of conducting this research is to investigate the effect of Ammonium addition to *Gracilaria* sp. on the absorption process of Mercury. The proportion amount of 10.5-gram *Gracilaria* sp. used in this research is mixed with 3 litres seawater media. The research method was performed using Completely Randomised Design (CRD) with four treatments and three replications. Parameters for the measurements are water quality, reduction of mercury and ammonium concentration, bio concentration factor, and growth rate. The research was designed with an addition of 0.05 ppm Mercury in all treatments, where each treatment was given different concentrations of Ammonium as follows: treatment A: 0.5 ppm, treatment B: 1 ppm, and treatment C: 1.5 ppm, with no addition of Ammonium for Control Treatment. The result of this research showed that treatment C hit the highest absorption level in absorbing Ammonium and Mercury with their concentrations of 95.98% and 56.44% respectively in seawater media, while the concentration of Mercury in the *Gracilaria* sp. biomass was measured 0.47 ppm.

Keywords: Absorption, Ammonium, *Gracilaria* sp., Phytoremediation, Mercury.

INTRODUCTION

Mercury is the most hazardous material since it contains non-essential metals that are not required by living beings, and also due to its toxicity in aquatic organisms (Hosseini *et al.*, 2016). Ministry of Environment's Decree No.51/2004 On Sea Water Quality Standards defined that the threshold value of the Mercury standard is 0.001 mg/l. Mercury contamination has been an issue for environment, biota, and even human health. The components of Mercury in the ocean, if converted by anaerobic microorganisms, will change to Methylmercury ((CH₃Hg)⁺) or Dimethylmercury (CH₃2Hg). Mercury is highly volatile and it is released from the seabed mud or sand to the water in the vicinity. Mercury ions are soluble in water, they accumulate in living organisms, and the network is bound to a protein. Mercury levels in marine environment should be monitored

regularly to check the water quality and ensure animal health (Hosseini *et al.*, 2016)

Bioremediation is the method used by organisms to clean up their polluted environment from pollutants. (Pribadi *et al.*, 2019) have carried out about bioremediation study to reduce ammonium contamination in water using bacteria called Microbial Fuel Cells (MFCs). The method of study was used a 30-day treatment for the catalysis of oxidation of inorganic and organic materials. Another study from Tanny (2006) conducted bioremediation by utilizing the plants called Phytoremediation. Phytoremediation can also be performed with the help of associations of plants such as microorganisms in order to degrade, withhold, or even change harmful contaminants that have been brought from their environment. The application of Phytoremediation principle is done by reducing the contaminants on the land

or sea where contamination is prevalent around shallow depths as long as they are still accessible by the phytoremediation agent (Paz-Alberto & Sigua, 2013). Macroalgae are selected as phytoremediation agent because of its abundance of high waters, with the activities of cultivation in particular. Macroalgae are part of eukaryotes that are highly potential to be the 'ecosystem engineers' since they provide organisms with a source of food and shelter, change the biotic into abiotic environment, and also build a habitat (Macreadie *et al.*, 2017). Macroalgae play a role as both bio-filter and bio-monitors that offer information about heavy metal concentrations (Khaled *et al.*, 2014). Research on macroalgae as bio-monitors of heavy metals pollution has been conducted by Bibak *et al.*, (2020), and the study showed that brown algae (*Padina gymnospora*) and red algae (*Hypnea hamulosa*) had the highest absorption level of heavy metals compared with green algae (*Cladophoropsis membranacea*). Another study from (Ihsan *et al.*, 2019) show that *Gracilaria verucosa* as a macroalgae can be used to absorb shrimp waste which becomes contaminants from culture such as nutrients heavy metals.

In Nuriwati (1985) study, *Gracilaria lichenoides* absorption against Mercury in the waters of the Bay of Jakarta provided a conclusion that Mercury can be absorbed as much as 0.01 ppm within eight weeks and the Mercury concentration of 0.005 ppm did not affect the growth of *Gracilaria* sp. Other studies with different pollutants (Pb) showed that *Gracilaria* sp. absorbed Pb up to 0 ppm from the planting mediums within 96 hours, with an addition of Ammonium as much as 0.5 ppm (Ihsan *et al.*, 2015). Until recently, there have only been a few studies that concern on the application of macroalgae as bio-monitors of heavy metals pollution. Therefore, this study aims to identify the impact of having Ammonium addition on the absorption of Mercury by Macroalgae *Gracilaria* sp.

MATERIALS AND METHODS

The research utilized experimental design of randomised complete (RAL) method with four treatments where each treatment was also repeated three times. The planting medium is 3-litre water, 10.5 grams of *Gracilaria* sp., and 0.05 ppm HgCl₂ as an addition. With the addition of NH₄OH and 0 ppm Control Treatment, treatments A, B, and C scored 0.5 ppm, 1 ppm, and 1.5 ppm respectively. Observation time was set to 0 hours, 12 hours, 24 hours, 48 hours, 72 hours, and 96 hours, whereas each sampling of *Gracilaria* sp. was

observed at the beginning and the end of the time of the study.

The Mercury (Hg) Concentration

The calculation of the absorbed Hg as heavy metal using the method of Langmuir equation showed as follows:

$$C_{\text{absorbed}} = C - C' \dots\dots\dots (1)$$

The Ammonium Concentration

The measurement of NH₄ concentration was calculated using Nessler method resulting in:

$$\frac{1000}{25} \times \frac{\text{Sample absorption}}{\text{Standard absorption}} \times 5\mu\text{g. ic} - 3 \dots\dots (2)$$

Absolute Growth

The Absolute Growth measurement is represented in the equation:

$$G = W_t - W_o \dots\dots\dots (3)$$

Description:

G = Absolute Growth (g); W_t = Weight at the end of the study (g); W_o = Weight during initial research (g)

Bio-concentration Factor (BCF)

BCF value of Hg as the heavy metal in *Gracilaria* sp. was calculated using the following formula (Walker *et al.*, 2012):

$$BFC = \frac{C_{\text{Hg organism}}}{C_{\text{Hg in Water}}} \dots\dots\dots (4)$$

BCF = Bio-concentration Factor; C = Metal Concentration (mg/kg); BCF >1 = accumulator plants; BCF →1 = indicator plants; BCF <1 = excluder plants.

RESULTS AND DISCUSSION

Ammonium Concentration at Growth Media

The Ammonium concentration in treatment A hit 4.07 ppm, while treatment B and treatment C scored 4.55 ppm and 5.06 ppm respectively. On the control medium, sea water was naturally prevalent without the addition of 3.47 ppm Ammonium concentration. Treatment C with its highest Ammonium concentration experienced a percentage decrease by 95.98% in concentration with an addition of 1.5 ppm Ammonium. Ammonium was needed by *Gracilaria* sp. and later utilised as nutrients (Table 1). Based on Table 1, *Gracilaria* sp. features essential characteristics, and one of them is their ability to absorb Ammonium as the source of nutrients. This fact shows that nutrients are still needed in a polluted environment.

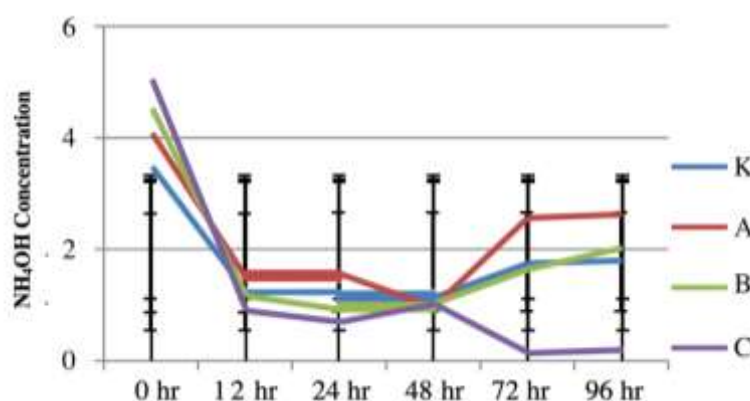
Table 1. Ammonium (NH₄OH) Concentration Reduction in Growth Media

Treatment NH ₄ OH	Average Concentration of NH ₄ OH (ppm)			Reduction (%)
	T ₀	T ₅	T ₀ - T ₅	
K	3.472	1.795	1.677	48.301 ^a
A	4.072	2.641	1.431	35.142 ^a
B	4.548	2.043	2.505	55.079 ^b
C	5.060	0.203	4.857	95.988 ^c

a = Significantly indifferent; b = Significantly different; c = Very significantly different

In Figure 1, the maximum decrease followed in the first twelve hours. Treatment A indicated that Ammonium increased in the following 72 hours. There is a possibility for Ammonium, that are stuck on the surface of the thallus to detach

themselves and return to planting media. Though both treatments B and C experienced similar fluctuations, during the 96-hour span, treatment C showed 0.203 ppm concentration of Ammonium absorption within the last hour.

**Figure 1.** Ammonium (NH₄OH) Concentration in Growth Media

The concentration decrease in the first twelve hours might have been attributed to acclimatisation when the hungry *Gracilaria* sp. felt the urge to directly absorb Ammonium as part of their nutrient intake. Absorption mechanism began from the absorption of the thallus on *Gracilaria* sp. followed by the absorption in the tissues and the whole process accumulated. Another possibility was nitrification, where oxidation of Ammonium occurred and turned them into Nitrite and Nitrate. Ammonium can be either contaminants or nutrients for living things. These findings showed, that in spite of the high absorption level of Ammonium reaching 95.98%, *Gracilaria* sp. still require Ammonium in a Mercury-polluted medium. The statistical analysis also showed

different concentrations of Ammonium experienced significant decrease.

Mercury (HgCl₂) Concentration on Media

During all treatments including Control Treatment, Mercury Chloride concentration was added and it was set to 0.05 ppm. The results showed, within the 96-hour maintenance period, Mercury ions experienced a maximum concentration decrease by 56.44% during treatment C, with the minimum decrease of 38.46% (**Table 2**). The statistics show no significant differences are found between Control Treatment and treatment B, though there are substantial differences amongst treatment A, treatment B, and treatment C.

Table 2. Mercury (HgCl₂) Concentration in Growth Media (ppm)

TreatmentHgCl ₂	Average Concentration of HgCl ₂ (ppm)			Reduction (%)
	T ₀	T ₅	T ₀ - T ₅	
K	0.0546	0.0336	0.021	38.462 ^a
A	0.0565	0.0299	0.027	47.080 ^b
B	0.0519	0.0306	0.021	41.040 ^a
C	0.0574	0.0250	0.032	56.446 ^c

a = Significantly indifferent; b = Significantly different; c = Very significantly different

The measurement results of the Mercury concentration within the 96-hour treatment

(**Figure 2**) show different levels of Mercury concentration decrease in each treatment. The

most significant decrease occurred in the first twelve hours. In Control Treatment, the concentration experienced an increase within 24 hours, but another decrease within the following 48 hours. Treatments A, B, and C continued to decline in fluctuation. The absorption mechanisms occurred in two stages,

namely active and passive. As heavy metal, Mercury entered through the plasma membrane, and other essential metals through the membrane transport system due to the similarity between the nature of Mercury and other essential metals.

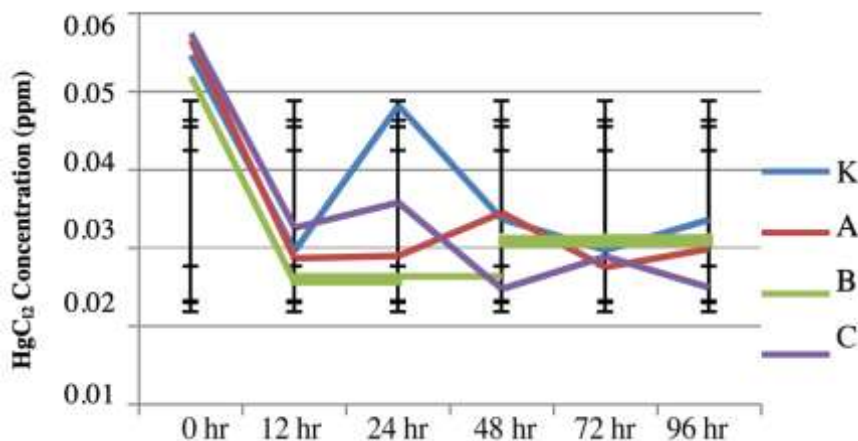


Figure 2. Mercury (HgCl₂) Concentration in Growth Media

Toxicological effects of mercury have known since a long time. The effects described depend on different parameters (dose and exposure route), yet they include abnormal on the organism organ (kidney, liver, lung), hematological alteration and given a neurological effect (Sweet & Zelikoff, 2001). Passive absorption occurs because Mercury ions get into contact with the cell walls, and a bond with the cell walls was created since proteins and polysaccharides bind them, turning toxic heavy metals to non-toxic compounds. Study from (Khangarot, 1981) reported about enormous variation in LC50 values for zinc at lower and higher temperature. The expanded sensitivity to hefty metals at higher temperature may be the aftereffect of expanded metabolic action, respiratory and heart rates, combined with temperature potentiation of metal particle activity on cell compounds and cell film.

The previous research showed that *Gracilaria* sp. had a rather high Mercury content amounting to 87.65%, creating a possibility for the cell walls to accumulate Mercury. However, since Mercury is heavy metal that is not required during the process of metabolism, more Ammonium than Mercury will be

absorbed by *Gracilaria* sp., apart from the fact that Ammonium Nitrogen happens to be the main macronutrients. Different absorptions of Mercury were shown in this study depending on the Ammonium concentrations added during the treatments. This shows that the absorption level of Mercury by Macroalgae is still menial since Mercury is considered non-essential metal that is not needed by *Gracilaria* sp. yet, a small percentage of Mercury is still absorbed due to *Gracilaria* sp.'s ability as super absorber plant. Based on the results of the statistical analyses, the decrease in the concentration of Mercury Chloride differs significantly.

Concentrations of Mercury (HgCl₂) in *Gracilaria* sp.

The study was performed, and statistically, the results have been observed to identify the difference of absorption levels of Mercury by *Gracilaria* sp. with 95% of confidence coefficient. *Gracilaria* sp.'s absorption ability of Mercury during Control Treatment differed significantly compared with other treatments, whereas during treatment C, *Gracilaria* sp.'s absorption of Mercury on its thallus differed quite considerably (Table 3).

Table 3. Result of Statistical Test for Mercury (HgCl₂) Absorption Data

Treatment	Concentration T0 (ppm)	Concentration T5 (ppm)
K	0.002	0.009 ^a
A	0.002	0.022 ^b
B	0.002	0.033 ^b
C	0.002	0.047 ^c

a = Significantly indifferent; b = Significantly different; c = Very significantly different

The addition of waste, like Mercury in particular, on the planting media was set to 0.05 ppm in all treatments. Difference treatments are performed based on each Ammonium's concentration. Figure 3 shows Mercury Chloride's contents with initial measurement of 0.002 ppm in the *Gracilaria* sp. during the early research after acclimatization and before the treatment. At the end of the research on Mercury Chloride's contents in *Gracilaria* sp.

the higher the amount of Ammonium is added, the higher the *Gracilaria* sp. can absorb Mercury Chloride through its body tissue. In general, the maximum permitted levels of Hg in different types of food are 0.5 ppm (BPOM). In all treatments, the levels of Mercury were still found below the permitted threshold value, yet treatment C showed that the Hg level almost hit the boundary set by the government.

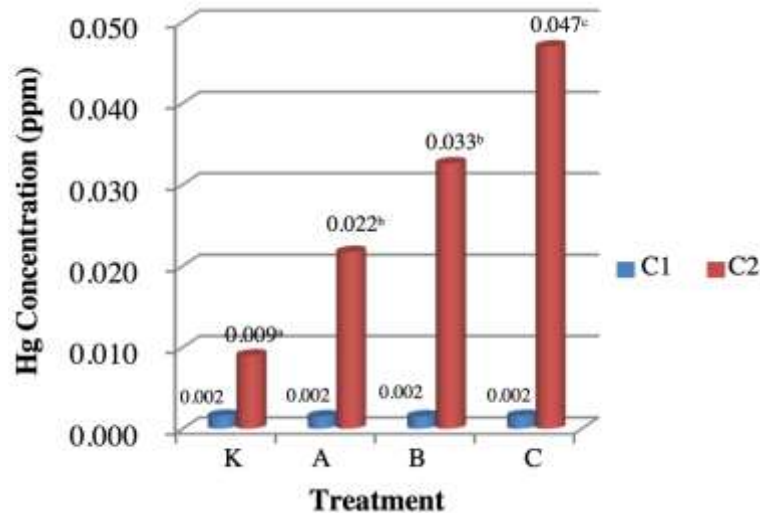


Figure 3. Absorption Capability of *Gracilaria* sp. in Mercury ($HgCl_2$)

Macroalgae possess the ability to absorb pollutants through their stem and create a bond with cytoplasm. On the stems of Macroalgae, cytoplasm when bound with non-essential metals can cause natural destruction of the cells' ability to do catalytic detoxification. The absorption process itself is influenced by the form of the thallus, and thallus that is located at the lower part with higher adherence to substrate has different absorption process from the one on the tip of thallus *Gracilaria* sp. (Serbula et al., 2013). The results showed that the greater the concentration of Ammonium is added, the higher the Mercury will be absorbed through the thallus of *Gracilaria* sp. and this illustrates that the Ammonium concentration affects Mercury absorption, and as shown in the statistics, the test results support this finding due to the prevalence of substantial differences.

The Growth of *Gracilaria* sp.

Initial weight measurements had been performed before all treatments so that each treatment had the same initial weight of 10.5 grams (Figure 4) and after 96 hours, macroalgae received the maximum weight of

10.75 grams in Control Treatment, while the lowest weight 10.36 grams was achieved in treatment A. A decrease in weight was experienced in treatment due to the many broken thallus of *Gracilaria* sp. that was also broken down because of insufficient oxygen intake. The decrease of *Gracilaria* sp. weight also in line with the high and low concentrations of heavy metal in the maintenance media (Yulianto et al., 2018). There was also another possibility where mercury ($HgCl_2$) inhibited the metabolic processes. The data examination to prove the growth significance showed that the data used for the proofing were significant with 95% of Confidence Coefficient. Nitrogen helped metabolism in a way that Mercury in the growing media could still be absorbed and *Gracilaria* sp. was still able to withstand pollutants in the growing media. Based on the results of the statistical analysis (Table 4), there has been no significant growth of *Gracilaria* sp. most likely due to the mediocre weight increase ranging between 0.25 grams and 0.12 grams. Several treatments such as light, temperature, nutrient supply, density and depth of *Gracilaria* sp. were also influence the growth of *Gracilaria* sp (Yang et al., 2006).

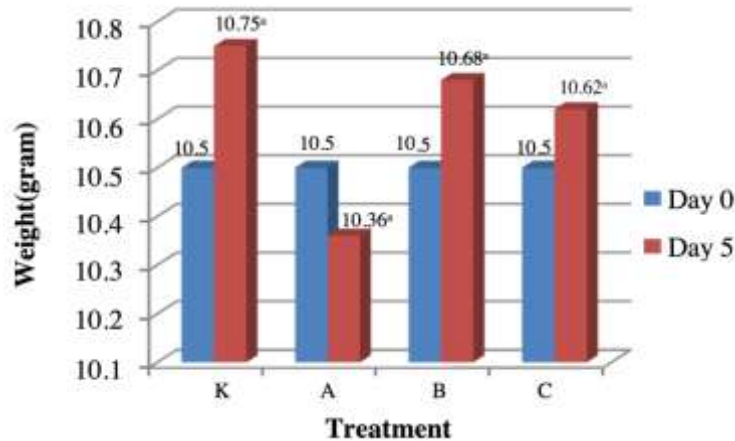


Figure 4. Growth of *Gracilaria* sp.

Table 4. Result of Statistical Test for *Gracilaria* sp. Final Mass

Treatment	Initial Live Weight (gram)	Final Weight (gram)
K	10.5	10.75 ^a
A	10.5	10.36 ^a
B	10.5	10.68 ^a
C	10.5	10.62 ^a

a = Significantly indifferent

Bio-Concentration of Hg in *Gracilaria* sp.

BCF values can be used to identify the level of ability in organisms to accumulate metals like Hg that enter into the body of *Gracilaria* sp. (Figure 5). The highest BCF values occurred in treatment C with 0.82 in value, while treatment B scored 0.63, treatment C 0.38, and Control Treatment 0.17. In treatment C, *Gracilaria* sp.

can be classified as Mercury pollution indicator plant with BCF value approaching 1. The obtained BCF values illustrate that *Gracilaria* sp. has the ability to be the indicator plant since *Gracilaria* sp. dominantly scored almost 1 (Walker *et al.*, 2012). *Gracilaria* sp. serving as the pollution bio-indicator due to the color change on the thallus.

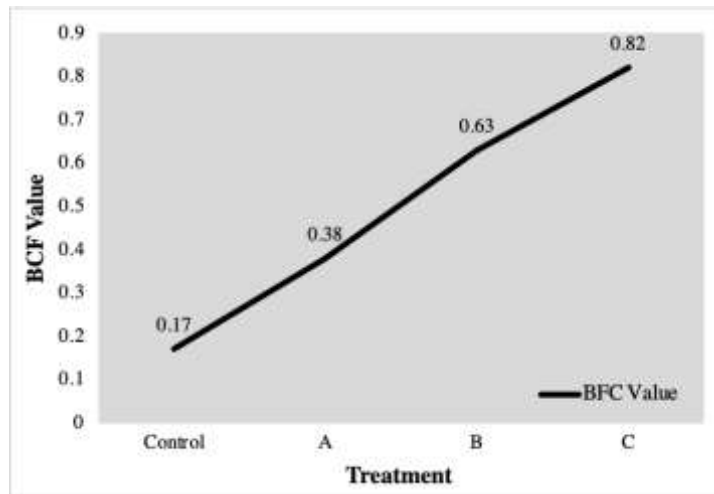


Figure 5. Mercury BCF on *Gracilaria* sp.

CONCLUSIONS and SUGGESTION

Based on the aforementioned research results and discussions, the addition of Ammonium Hydroxide indeed interferes the ability of *Gracilaria* sp. in absorbing Mercury Chloride as it has been demonstrated where the higher Ammonium concentration is added, the higher

Mercury Chloride will be absorbed by *Gracilaria* sp. reaching 56.44%. The maximum absorption of Ammonium Hydroxide and Mercury Chloride reaches its highest level within the first twelve hours. Statistically, the test results showed a significant difference in the absorption of Ammonium Hydroxide and Mercury Chloride, though not significant when compared to the

weight of *Gracilaria* sp. itself. With 1.5 ppm of Ammonium Hydroxide addition in treatment C, Macroalgae *Gracilaria* sp. can be classified as indicator plant scoring almost 1 (0.82) in BCF value.

ACKNOWLEDGEMENTS

This study was conducted by Universitas Padjadjaran, which was cooperatively conducted by Indonesia Directorate General of Higher Education. We thank to the members of the Department of Marine Science, Universitas Padjadjaran for their tireless efforts during a large and long experiment. We appreciate the willingness of Faculty of Fisheries and Marine Sciences, Padjadjaran University to provide samples although ultimately their area of coverage was outside the range of this study.

REFERENCES

- Bibak, M., Sattari, M., Tahmasebi, S., Agharokh, A., & Namin, J. I. (2020). Marine macro-algae as a bio-indicator of heavy metal pollution in the marine environments, Persian Gulf. *Indian Journal of Geo-Marine Sciences*, 49(3), 357–363.
- Hosseini, M., Nabavi, S. M. B., Parsa, Y., & Saadatmand, M. (2016). Mercury contamination in some marine biota species from Khuzestan shore, Persian Gulf. *Toxicology and Industrial Health*, 32(7), 1302–1309. <https://doi.org/10.1177/0748233714555392>
- Ihsan, Y. N., Aprodita, A., Rustikawati, I., & Pribadi, T. D. K. (2015). The ability of *Gracilaria* sp. as a bioremediation agent in absorbing heavy metal Pb. *Jurnal Kelautan*, 8(1), 10–18.
- Ihsan, Y. N., Subiyanto, Pribadi, T. D. K., & Schulz, C. (2019). Nitrogen assimilation potential of seaweed (*Gracilaria verrucosa*) in polyculture with pacific white shrimp (*Penaeus vannamei*). *AACL Bioflux*, 12(1), 51–62.
- Khaled, A., Hessein, A., Abdel-Halim, A. M., & Morsy, F. M. (2014). Distribution of heavy metals in seaweeds collected along marsa-matrouh beaches, Egyptian mediterranean sea. *Egyptian Journal of Aquatic Research*, 40(4), 363–371. <https://doi.org/10.1016/j.ejar.2014.11.007>
- Khangarot, B. S. (1981). Lethal Effects of Zinc and Nickel on Freshwater Teleosts. *Acta Hydrochimica et Hydrobiologica*, 9(3), 297–302. <https://doi.org/10.1002/aheh.19810090308>
- Macreadie, P. I., Jarvis, J., Trevathan-Tackett, S. M., & Bellgrove, A. (2017). Seagrasses and Macroalgae: Importance, Vulnerability and Impacts. In *Climate Change Impacts on Fisheries and Aquaculture* (Issue November). <https://doi.org/10.1002/9781119154051.ch22>
- Paz-Alberto, A. M., & Sigua, G. C. (2013). Phytoremediation: A Green Technology to Remove Environmental Pollutants. *American Journal of Climate Change*, 02(01), 71–86. <https://doi.org/10.4236/ajcc.2013.21008>
- Pribadi, T. D. K., Ihsan, Y. N., Fellatami, K., Syamsuri, R. R. P., & Koswara, B. (2019). Microbial Fuel Cell: Sustainable Approach for Reservoir Eutrophication. *Asian Journal of Water, Environment and Pollution*, 16(1), 1–8. <https://doi.org/10.3233/AJW190001>
- Serbula, S. M., Kalinovic, T. S., Ilic, A. A., Kalinovic, J. V., & Steharnik, M. M. (2013). Assessment of airborne heavy metal pollution using *Pinus* spp. and *Tilia* spp. *Aerosol and Air Quality Research*, 13(2), 563–573. <https://doi.org/10.4209/aaqr.2012.06.0153>
- Sweet, L. I., & Zelikoff, J. T. (2001). Toxicology and Immunotoxicology of Mercury: *Journal of Toxicology and Environmental Health, Part B*(4), 161–205.
- Walker, C. H., Sibly, R. M., Hopkin, S. P., & Peakall, D. B. (2012). *Principles of Ecotoxicology* (fourth). CRC Press Taylor & Francis Group.
- Yang, Y. F., Fei, X. G., Song, J. M., Hu, H. Y., Wang, G. C., & Chung, I. K. (2006). Growth of *Gracilaria lemaneiformis* under different cultivation conditions and its effects on nutrient removal in Chinese coastal waters. *Aquaculture*, 254(1–4), 248–255. <https://doi.org/10.1016/j.aquaculture.2005.08.029>
- Yulianto, B., Pramesti, R., Hamdani, R., Sunaryo, S., & Santoso, A. (2018). Biosorption Capacity and Growth of Seaweed *Gracilaria* sp. In Media Containing Heavy Metal Cadmium (Cd). *Jurnal Kelautan Tropis*, 21(2), 129. <https://doi.org/10.14710/jkt.v21i2.3849>