Research Inventy: International Journal of Engineering And Science
Vol.6, Issue 5 (May 2016), PP -28-33
Issn (e): 2278-4721, Issn (p):2319-6483, www.researchinventy.com

Bioaccumulation of Cadmium Heavy Metal And its Effect on the Level of Chlorophyll And Carotenoids of Thalassia Hemprichii in the Waters of Ambon Island

[1]Prelly M.J Tuapattinaya, [1,*]Dominggus Rumahlatu, [2]Stenly Tulalessy
[1]*Biology Education Study Program, Faculty of Teacher Training and Education Science, Pattimura University, Indonesia*
[2]*Graduate Students of Biology Education Study Program, Faculty of Teacher Training and Education Science, Pattimura University, Indonesia*

Abstract : *Cadmium is known to inhibit the biosynthesis of chlorophyll and can disrupt the photosynthesis process. This research aimed at investigating the ability of the accumulation of cadmium heavy metals (Cd) by Thalassia hemprichii in the waters of the Ambon Island and its effect on the levels of chlorophyll and carotenoid in the leaves. Cd heavy metal analysis was carried out on samples of sea water, sediments, as well as the roots and leaves of T. hemprichii using Atomic Absorption Spectrometer (AAS). The ability of accumulation and translocation of Cd heavy metals T. hemprichii was determined by calculating the Bioconcentration Factor (BCF) and translocation factor (TF). The analysis of chlorophyll and carotenoid content used UV-Vis Spectrophotometer. The results of the research showed that the BCF value of T. hemprichii reached 141.04, while the TF value was 7.63. The BCF and TF value which was more than one indicated that T. hemprichii had the potential to be metal accumulators. The effect of the levels of Cd heavy metals in the leaves and the level of chlorophyll showed a negative correlation, but not significant. The level of Cd heavy metals in the leaves also could increase the levels of carotenoids of the leaves of T. hemprichii. These results indicate that T. hemprichii had the potential as phytoremediator in the waters of Ambon island that have been contaminated with heavy metals Cd.*

Keywords: *Heavy metal cadmium, Thalassia hemprichii, chlorophyll, carotenoids*

I. Introduction

Cadmium metal is a dangerous heavy metal which, if accumulated in humans, may cause disease and harmful abnormality including lung cancer and some types of cancer, renal dysfunction, osteoporosis, respiratory problems and reduce the number of births [1].

Cadmium metal is a nonessential element for plants and can easily be accumulated by plants. The intake of cadmium ion by plants is done through the channel protein transmembrane of the other nutritional elements such as K, Ca, Mg, Fe, Mn, Cu, Zn, Ni [2]. This Heavy metal has high toxicity effects and has a negative effects on the growth and development of plants, transpiration, regulation of stomata, the enzymatic activity, water absorption, the absorption of elements/essential minerals, protein metabolism and membrane function [3]. Chlorosis, a shortage of chlorophyll in plants, is also one of the consequences caused by the accumulation of cadmium. The heavy metal cadmium can inhibit the absorption of nitrogen (N), so that it may cause chlorosis [4].

Chlorosis or the shortage of chlorophyll is the primary symptom for plants which are exposed to high levels of cadmium [5]. Chlorosis can occur due to deficiency of iron (Fe), phosphorus (P) or reduce the transporter of mangan element (Mn) [2].

Cadmium is known to inhibit the biosynthesis of chlorophyll by reacting with thiol groups in the process of synthesis of 5-aminolevulinic acid and the enzyme complex of protocholrophyllide reedukstase. Cadmium can also substitute for Mg^{2+} ion from the chlorophyll molecules that can disrupt the photosynthesis process [6]. Carotenoids are a group of isoprenoid molecule that has many important functions for the plant such as stabilizing the lipid membrane, protecting the photosystem of fotooxidasi, precursor to the ABA hormone (abisisat acid) and also give color to fruits and flowers to invite pollinator [7]. The main function of carotenoid is to play a role in the protection system against oxidative damage. These pigments will interact with molecules of *Reactive Oxygen Species* (ROS) that is the *singlet oxygen* ($^1O2$) and *hydrogen peroxide* (H_2O_2) molecular [8]. The cadmium which accumulates can lead to *reactive oxygen species* (ROS), which would cause oxidative effects on cells. Cadmium is a heavy metal non-redox, but it can induce various types of ROS, including *superoxide radical* ($O_2^{*\cdot}$), *hydrogen peroxide* (H_2O_2) and *hydroxyl radical* (OH*) that can cause cell apoptosis and or the other oxidative effects, such as protein oxidation, enzyme inhibition, membrane lipid peroxidation and nucleic acid damage [4].

In the waters of Ambon Island, cadmium accumulates in the water and sediment. Metal cadmium in the aquatic environment comes from community activities such as industry, ship repair, ship loading and unloading of pertamina oil and sea transportation, and it was also assumed to have come from the natural processes such as abrasion of the river [9].

Seagrass are a group of flowering plants which are unique and can adapt well on the marine environment. Seagrass can grow in the intertidal and subtidal zones provided that these waters are still exposed to sufficient light [10]. *Thalassia hemprichii* is an aquatic organism, so that it is easily exposed to the metal cadmium. *Thalassia hemprichii* is able to accumulate cadmium metal very well. With these capabilities, it is expected that seagrass species spreading across the Ambon Island has accumulated cadmium metal [11].

The ability of the cadmium heavy metal accumulation by *T. Hemprichii*was seen from the value of bioconcentration factor/bioconcentration factor (BCF), that is, the comparison between the levels of heavy metals in the root/leaf of seagrass and the surrounding water/sediment. The ability of translocation of heavy metals was known from the value of the translocation factor (TF),that is the comparison between the heavy metals in the leaf tissue and that in the roots of the *T. Hemprichii* sea grass. Based on the background that has been presented, this research is important to investigate the ability of heavy metal bioaccumulation and its effects on the levels of chlorophyll and carotenoid of *T. hemprichii* in the waters of Ambon island.

II. Materials And Methods

2.1 Research sampling

The samples used in this research were sea water, sediments and *T. hemprichii.* Samples were taken from two different intertidal locations namely coastal waters of Galala village as Station I and coastal waters of Hutumuti Village as the station II. The sample of water was taken using a clear glass bottle which was then given drops of HNO_3 until the pH became 2. The sample of sediment was taken using PVC pipe until 10 cm. The sediments taken was then put into a plastic bag. The sample of seagrass *T. hemprichii* was taken by *purposive sampling*, that was the seagrass *T. hemprichii* with leaves 12-15 cm long. The seagrass samples were taken as many as 10 individuals/stations. In station I, the sampling was done in three locations of sampling points. The locations of sampling points were divided based on the location of the sampling point to the coastline. The first, the second and the third sampling points were 0.5 and 10 m from the shoreline at low tide. At the station II, however, seagrass T. hemprihii was taken at only one sampling point with the distance of 5 meters from the shoreline at low tide. The seagrass samples that had been collected were then put into an *ice box* and taken to a laboratory to measure the levels of heavy metals, carotenoids and chlorophyll of the leaves of *T. hemprichii.*

2.2 Determination of the heavy metal cadmium in the samples

The water samples were then measured for the levels of the cadmium heavy metals. The sediment samples were then dried in the oven, and then the levels of the metal cadmium were measured. The steps of determining the levels of the metal on the samples of seagrass are described as follows. The samples of *T. hemprichii* were cleaned with distilled water and then the roots and the leaves of the *T. hemprichii* were separated. The samples of roots and leaves were equally treated. They were cut into small pieces and dried using a preheated oven at 105^0C for 20 hours and followed with a temperature of 80^0C for 72 hours in order to equalize the sample weight. After that, the sample was crushed and weighed 500 mg. Then, sample was digested with HNO_3 solution-$HClO_4$ and heated to a temperature of 100^0 -200^0 C until white steam came out, and its volume was measured. After that, the samples of roots and leaves of *T. hemprichii* were measured using *Atomic Absorption Spectrometer* (AAS) [11].

After the level of cadmium metal was found out by the AAS, the data were then used to calculate the level of metal content in tissues (Cy ') *T. hemprichii* [12].

$$Cy' = Cy \times \frac{V}{W}$$

Cy '= metal content in plant tissues (ug/g)
Cy = metal concentrations measured in AAS (mg/mL)
V = dilution volume (mL)
W = dry tissue weight (g)

To calculate Bioconcentration factor (BCF), using the equation [13] as follows.

$$BCF = \frac{\text{the heavy metal Cd in roots or leaves}}{\text{the heavy metal Cd in sediment or water}}$$

To calculate the translocation factor (TF) of *T. hemprichii* using equation [3] as follows.

$$TF = \frac{the\ heavy\ metal\ Cd\ in\ leaves}{\text{the heavy metal } Cd\ in\ roots}$$

2.3 The determination of levels of chlorophyll and carotenoids of the leaves of *Thalassia hemprichii*

Samples *T. hemprichii* from each station was previously washed and taken to determine the chlorophyll level of the leaves. The leaves used were the second or the third leaf counted from the base. After that, it was weighed with a weight of 0.1 mg. The samples were then homogenized using mortal and acetone 85% 10 mL was added with a ratio of the sample weight: acetone is 1:100 in dark room. And then, it was centrifuged with the speed at 4000 r/min at 4^0C for 5 minutes. And then it was filtered with a filter paper and the filtrate was analyzed using UV-Vis spectrophotometer at a wavelength of 646 nm, 663 nm and 480 nm [11]. After the absorbance values was obtained, the chlorophyll content (mg/L) was determined by the formula:

Chlorophyll a = 12.7 (A_{663}) - 2,67 A_{646})
Chlorophyll b = 22.9 (A_{646}) - 4.68 (A_{663})
Chlorophyll total = 20.2 (A_{646}) + 8.02 (A_{663})

To calculate the amount of carotenoids used the following formula:

$$Caretenoids = \frac{1000\ (A480) - 1{,}43\ \ KloA - 35{,}87\ KloB}{205}$$

III. Data Analysis

To assess the ability of heavy metal accumulation in roots and leaves of *T. hemprichii*, the data were analyzed descriptively. To determine the effect of the metal content of cadmium on the levels of chlorophyll and carotenoid, the data were analyzed using the equation of simple linear regression Y = ax + b, with Y is the dependent variable, namely the levels of chlorophyll and carotenoids whereas x is the independent variable, the level of metal cadmium Cd on the tissue of *T, hemprichii.*

IV. Results And Discussion

4.1 Bioaccumulation of Heavy Metal Cadmium By Seagrass *Thalassia hemprichii*

The results showed that there was an accumulation of cadmium metal in roots and leaves of seagrass *T. hemprichii* (Table 1). At station 1, the accumulation of heavy metals in the roots of *T. hemprichii* ranged from 0.92-0.99 ppm, while the level of cadmium metal of the leaves of *T. hemprichii* ranged from 0:24 to 1:17 ppm. At station II, the levels of cadmium heavy metal in the roots and leaves of *T. hemprichii* was 0:12 and 0.91. The data showed that seagrass *T. hemprichii* could accumulate cadmium heavy metals from the surrounding waters. Cadmium metal was significantly higher in the seagrass *Posidonia ocenica* compared to that of the scallop *Mytilus galloprovincialis* types [14].

Table 1 Levels of Cadmium Heavy Metals in Water, Sediment and *Thalassia hemprichii*

Sampling Points		Levels of Cadmium Heavy Metals (ppm)			
		Sea water	sediment	*T. hemprichii*	
				Root	Leaf
Station I: Village Galela	I	0.07	0.01	0.96	0.24
	II			0.99	0.42
	III			0.92	1.17
Station II: Village Hutumuri		0.09	0.12	0.12	0.91

The ability of cadmium metal accumulation by *T. hemprichii* could be quantitatively counted with the value of bioconcentration factor (BCF), while the ability of cadmium metal translocation from the roots up to the leaves could be determined by the value of *translocation factor* (TF). BCF and TF value of seagrass *T. hemprichii* (Table 2).

The ability of bioaccumulation *T. hemprichii* can be seen from the value of Bioconcentration factor (BCF), where there was a comparison of the concentration accumulated by *T. hemrichiii* and the heavy metals contained in the sediments. BCF value at both stations showed that *T. hemprichii* had the potential as the metal accumulators. It was found that the value of BCF from each village station was more than 1. In the village station I, the BCF value of seagrass *T. hemprichii* reached 141. While at station II, the BCF value was 7.48. BCF value > 1 indicates that plants are potential as heavy metal cadmium accumulators [15].

Table 2 Value of Bioconcentraton Factor (BCF) and Translocation Factor of (TF) Seagrass *Thalassia hemprichii* on Cadmium Heavy Metal

Sampling Points		BCF	TF
Station I: Galela Village	I	136.84	0.25
	II	141.04	0.42
	III	131.13	1.27
Station II: Hutumuri Village		7:48	7.63

The cadmium contained in seagrass tissue *T. hemprichii*, *Enhalus acoroides* and *Cymodocea rotundata* came from the sediments absorbed by the roots. The cadmium heavy metal entered the roots if seagrass *T. hemprichii* through essential metal chanel and accumulated by a the tissue of plant roots. After accumulated in roots, the heavy metal was translocated to the other organs of the plants [16]. This translocation capability can be seen by knowing the value of translocation factor (TF) of the plant. Heavy metals moved from the roots to the top through transpiration of plants (xylem), level element transportation are not the same on each element and each type of plant [15].

The TF value of *T. hemprichii* station I quite varied, namely from 0.25 to 1.27. Station II had a value of 7.63 TF. The TF value of *T. hemprichii* on both stations showed that kadmum metal translocation ability from the roots to the leaves *of T. hemprichii* was high. These capabilities showed that *T. hemprichii* is a metal accumulator plant which is pretty good. The TF value > 1 indicated that the plant had a metal concentration in leaves higher than that in roots, and it is the characteristic of hyper accumulator plants. Hyper accumulator plants are plants that can accumulate the metal with a very high concentration in the surface tissue *(above ground)* [17].

The high translocation in the leaves of *T. hemprichii* was expected because seagrasses had many ligands and capable to be translocated to the leaves along with the other essential elements through the xylem. In Indian mustard, cadmium can be translocated to the leaves by binding to phytokelatin as ligands [18]. In addition, heavy metals that enter the body of the plant will experience a variety of processes as a response of the plants to cope with the toxic material in the body, coping mechanisms that may occur is localization, excretion, dilution to weaken the toxic effects of heavy metals through dilution and chemical inactivation [19].

4.2 The levels of Carotenoid and Chlorophyll of *Thalassia hemprichii*

Cadmium heavy metal accumulated by organisms will cause a variety of physiological effects. The effects of cadmium heavy metals on the levels of chlorophyll and carotenoids of seagrass *T. hemprichii* can be seen in Figure 1. At station II, the level of metals on the tissue of the leaves was 0,91 ppm having the levels of chlorophyll a, b and the total of each was 2.56 mg/g; 2.85 mg/g and 5.4mg/g and the carotenoid content of 0,49 mg/g. While at station II, the levels of chlorophyll "a" ranged between 5.2 and 6.25 mg/g, chlorophyll "b" ranged between 5.1 and 5.69 mg/g, the total chlorophyll ranged from 10.46 to 11.35 mg/g.

The correlation between the levels of chlorophyll a, b and the total contained in the leaves of *T. hemprichii* and the level of cadmium metal in the leaves at both stations showed a negative correlation, although the levels of chlorophyll of *T. hemprichii* showed a tendency of decrease with the increase in the concentration of cadmium in leaves, but the coefficient values of the correlation was small (chlorophyll a, b and total with R = 0,013; R=0,236; R=0,056). Therefore, it can be said that at the concentrations of 0,24 to 1,17 ppm, cadmium did not have a significant effect on the level of chlorophyll in the leaves of *T. hemprichii.*

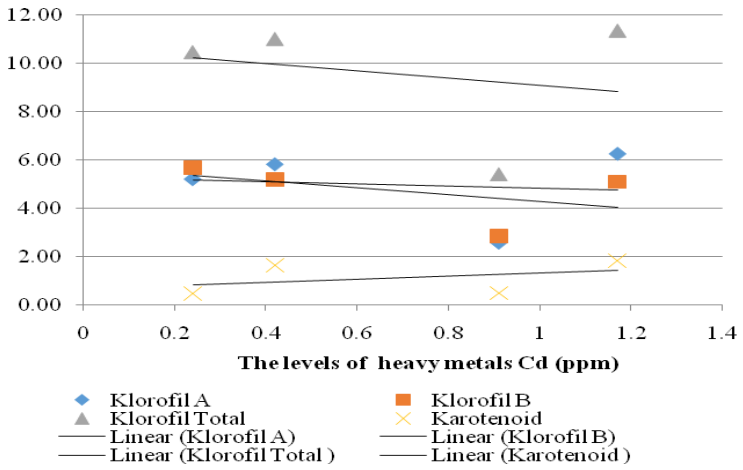

Fig 1 The correlation between Heavy Metal level and the level of Chlorophyll A, B, Total and Carotenoids of the leaves of *Thalassia hemprichii*

Previous research showed that exposure to cadmium could reduce the levels of chlorophyll a and b in seagrass *T. hemprichii* [11]. The other research findings showed that the *Posidonia oceanica,* the total level of chlorophyll decreased significantly, while the chlorophyll a and b were not too different from the control [20]. The level of chlorophyll also decreased in *Zea mays* and *Triticum aestivum* [3] and *Brassica juncea* [21] after exposed to metal cadmium. This decrease was because the cadmium metal may interfere the absorption, transport and the use of some important elements that play a role in the synthesis of chlorophyll, namely calcium, magnesium, phosphorus and potassium. Cadmium also reduced the absorption of nitrate and the transport from the roots to the top by inhibiting the activity of the nitrate reductase enzyme in the top [2].

In this research, it appeared that there was a decline in the level of chlorophyll, but it was not very significant. It was assumed that at the concentration of cadmium metal 1.17 ppm, *T. hemprichii* was still tolerant. If compared to other plants, a concentration of 1 ppm is a toxic dose for plants [22]. The tolerance of cadmium heavy metals was assumed to be the results of the localization ability of cadmium metal in the vacuole of leaves and the capabilities of cadmium ligand such as fitokelatin (Pc) owned by *T. hemprichii.* The associations of cadmium heavy metal with ligand can reduce the effects of toxic cadmium metal that can reduce the levels of chlorophyll of *T. hemprichii.* Fitokelatin proved to be instrumental in the mechanisms of plant tolerance to heavy metals [23, 24, 25]. Besides it was assumed that the tolerance of *T. hemprichii* to the cadmium metal was because it had carotenoid pigments and other oxidative enzymes that played a role in reducing the oxidative effects of cadmium heavy metal.

The research results showed that the level of cadmium in leaves had a positive correlation with the leaf carotenoids. This showed that the increase of cadmium heavy metal in the leaves was also accompanied by an increase in the carotenoid pigments. Previous research was also able to identify a significant increase in carotenoids after the exposure to heavy metals including cadmium on seagrass *T. hemprichii* [11] and *Brassica juncea* [21].

The Increased level of carotenoids was assumed to be from the ROS-induced by the accumulation of cadmium. ROS which acted as a signaling molecule inducing cells to synthesize carotenoids as a defense mechanism in dealing with the effects of oxidative nonenzimatik. Carotenoids are the first defense line against *singlet oxygen.* Carotenoids have a special chemical structure that is able to neutralize or extinguish the *singlet oxygen* reactivity and to provide energy to the entire carotenoid molecule. To be able to extinguish the *singlet oxygen,* carotenoids must have at least 9 double bonds with single bonds between the double bonds. This chemical composition is called a double bond conjugation. The energy of *singlet oxygen* was transferred to the carotenoid and returned to its original energy level. At the time, *singlet oxygen* has been converted into normal oxygen. In addition to *singlet oxygen,* the other kinds of ROS molecules can be neutralized by the carotenoids via electron transfer reactions, the formation of radical cluster formation or by transferring hydrogen atom. Thus, the carotenoids will increase concurrently with the increase of the cadmium heavy metal to prevent the damaging effects that can be caused by metal cadmium [26].

This research showed that *T. hemprichii* had the potential to remediate an environment contaminated with cadmium heavy metals because it was found that the *T. hemprichii* was able to accumulate and translocate cadmium heavy metal to the tissue in the top (leaves), the accumulation of cadmium heavy metals was high in the leaves but it did not significantly affect the level of the leaf chlorophyll and It has the anti-oxidative mechanisms against heavy metal that is by increasing the levels of carotenoids of the leaves. The ability of *T. hemprichii* is expected to play a role in reducing cadmium heavy metal pollution in the waters of Ambon island.

V. Conclusion

T. hemprichii is able to absorb cadmium heavy metals from aquatic environments with the ability of bioconcentration factor of 141 and translocation factor of 7.47. This value is more than 1indicating that the *T. hemprichii* in Ambon island waters is capable of absorbing cadmium metals and translocate it to the leaves, so that it is potential as phytoremediator. In addition, the results of this research showed that the cadmium heavy metal could reduce the level of leaf chlorophyll of *T. hemprichii,* but increasing the levels of carotenoids of the leaves of *T. hemprichii.*

Acknowledgement

The author is very grateful to the Dean Faculty of Teacher Training and Education Science, Pattimura University, who had provided the funding of this research through the collaborative research program Lecturer-Student budgetary year 2015, so that we can conduct the research and write this article. The author would like to thank the reviewer who had proofread this article so that it could be published.

References

[1]. Tvermoes, B.E., G.S. Bird, and J.H. Freedman. Cadmium Indued Transcription Independent On Intercellular Calcium Mobilization. Plos One, 6(6), 2011, 1-11

[2]. Benavides, M.P., S.M. Gallego, and M.L. Tomaro. Cadmium Toxicity In Plants. Brazil Journal Plant Physiology, 17, 2005, 21-34.

[3]. Nikolic, N., M. Borisev, S. Pajevic, and M. Zupunski. Responses Of Wheat (Triticum aestivum L.) And Maize (Zea mays L.) Plants To Cadmium Toxicity In Relation To Magnesium Nutrition. Acta Bot, Croat, 73(2), 2014, 359-373.

[4]. Gill, S.S and N. Tuteja. Cadmium Stress Tolerance In Crop Plants. Plant Signaling & Behavior, 6(2), 2011, 215-222.

[5]. Benabid, H & M.F. Ghorab. Is The Decrease Of Chlorophyll First Alert Of Pollution? A Study Of The Effect Of Cadmium In Chlorophyll Content Of Candian Wonder Beans (Phaseolus vulgaris). Report On International Conference And Chemical, Civil, and Enviroment Enginering Dubai, 2012, 279-282.

[6]. Gill, S, S., N.A. Khan, A and N. Tuteja. Differential Cadmium Stress Tolerance In Five Indian Mustard (Brassica juncea L.) Cultivars. Plant Signaling & Behavior, 6(2), 2011, 293-300.

[7]. Zhang, L., G. Ma, M. Kato, K. Yamawaki, T. Takagi, Y. Kiriiwa, Y. Ikoma, H. Matsumoto, T. Yoshioka, and H. Nesumi. Regulation Of Carotenoid Accumuulation and The Expression Of Carotenoid Metabolic Genes In Citrus Juice Sacs In Vitro. Journal Of Experimental Botany, 62(2), 2012, 871-886.

[8]. Gomez-Garcia, M.D.R., and N. Ochoa-Alejo. Biochemistry and Molecular Biology Of Carotenoid Biosynthesis in Chili Papppers (Capsicum spp.). International Journal Molecular Science, 14, 2013, 19025-19053.

[9]. Rumahlatu, D. Konsentrasi Logam Berat Kadmium Pada Air, Sedimen dan Deadema setosum (Echinodermata, Echinodea) Di Perairan Pulau Ambon (Concentrations of Cadmium Heavy Metal On Water, Sediment and Deadema setosum (Echinodermata, Echinodea) in the waters of Ambon island). Jurnal Ilmu kelautan, 16(2), 2011, 78-85.

[10]. Orth, R.J., T.J.B. Carruthers, and W.C. Dennison. A Global Crisis For Seagrass Ecosystems. BioScience, 56(12), 2006, 987-996.

[11]. Lei, L., H. Xiaoping, D. Borthaku, and N. Hui. Photosytheti Activity And Antioxidatif Respons Of Seagrass Thalassia hemprichii To Trace Metal. Acta Oceanol, 31(3), 2012, 98-108

[12]. Sembiring, E., and E. Sulistyawati. Akumulasi Pb dan Pengaruhnya pada Kondisi Daun Swietenia macrophyla King (Accumulation of Pb and Its Effect on Conditions of leaves of Swietenia macrophyla King). Proceedings of the Seminar Nassional Environmental Research ITB, 2006, (Online) (http://www.sith.itb.ac.id/profile/databuendah/Publications/ 7.%20Ebinthalina_IATPI2006.pdf) (Accessed on October 27, 2014).

[13]. Puspita, A.D., Santoso, and B. Yulianto. Studi Kandungan Logam Timbal (Pb) Dan Efeknya Terhadap Kandungan Klorofil Daun Mangrove Rhizopora mucronata (Study of Lead metal content (Pb) and its effect on Chlorophyll Content of Leaves of Mangrove Rhizophora mucronata). Journal of Marine Research, 3 (1), 2013, 44-53.

[14]. Lafabrie, C., G. Pergent, R. Kantin, C. Martini-Pergent, and J.L. Gonzales. Trace Metals Assessment In Water, Sediment, Mussel and Seagrass Species – Validation Of The Use Posidonia oceanica As A Metal Biomonitor. Chemosphere, 68(11), 2007, 2033-2039.

[15]. Suswana, R., and D. Suswati. Bioakumulasi Dan Distribusi Cd Pada Akar Dan Pucuk 3 Jenis Tanaman Famili Brassicaceae: Impelemnetassinya Untuk Fitoremediasi (Bioaccumulation And Distribution of Cd On Roots and Shoots of 3 Family Brassicaceae Plant types: the implementation for Phytoremediation). Jurnal Manusia dan Lingkungan, 20(2), 2013, 221-228.

[16]. Lei, L., and H. Xiaoping. Three Tropical Seagrassses As Potential Bio-indicators To Trace Metals In Xincun Bay, Hainan Island, South China. Chinesse Journal Of Oceanology And Limnology, 30(2), 2012, 212-224

[17]. Siahaan, M.T.A., Ambariyanto and B. Yulianto. Pengaruh Pemberian Timbal (Pb) Dengan Konsentrasi Berbeda Terhadap Klorofil, Kandungan Timbal Pada Akar Dan Daun, Serta Struktur Histologi Jaringan Akar Anakan Mangrove Rhizophora mucronata (the Effect of Lead (Pb) With Different Concentrations on Chlorophyll, Content of Lead On Roots And Leaves, Histology Structure of Root tissue of Mangrove Rhizophora mucronata). Journal Of Marine Research, 2(2), 2013, 111-119.

[18]. Gong, J., A.D. Lee, and Schoroeder. Long-distance Root-to-shoot Transport Of Phytochelatins And Cadmium In Arabidopsis. PNAS, 100(17), 2003, 10118-10123

[19]. Handayani, T. Bioakumulasi Logam Berat Dalam Mangrove Rhizophora mucronata Dan Avicennia marina Di Muara Angke Jakarta (Bioaccumulation of Heavy Metals In Mangrove Rhizophora mucronata and Avicennia marina in Muara Angke, Jakarta). Jurnal Teknik Lingkungan, 7(3), 2006, 266-270

[20]. Greco, M., A. Chiappetta, L. Bruno, and M.B. Bitonti. In Posidonia ocenica Cadmium Induces Change In DNA Methylation And Chromatin Patterning. Journal Of Experimental Botany, 63(2), 2012, 695-709

[21]. Kapoor, D., S. Kaur, and R. Bhardwaj. Physiological And Biochemical Change In Brassica juncea Plant Under CD-Induced Stress. BioMed Research International, 2014, 1-13.

[22]. Widyati, E. Potensi Tumbuhan Bawah Sebaga Akumulator Logam Berat Untuk Membantu Rehabilitasi Laahan Bekas Tambang (Potential of Bawah plant as an Accumulator of Heavy Metals Used to Assist Rehabilitation of former Mine site). Mitra Hutan Tanaman, 6(2), 2011, 46-56.

[23]. Wawrzynski, A., E. Kopera, A. Wawrzynska, J. Kaminska, W. Bal, and A. Sirko. Effect Of Simultaneous Expression Of Heterologous Genes Involved In Phytochelatin Biosynthesis On Thiol Content And Cadmium Accumulation In Tobacco Plant. Journal Of Experminetal Botany, 57(10), 2006, 2173-2182.

[24]. Wojas, S., S. Clemens, J. Hennig, S.A. Odowska, E. Kopera, H. Schat, W. Bal, and D.M. Antosiewiz. Overexpession Of Phytochelatin Synthase In Tobacco Distintive Effect Of AtPS1 and CePCS Genes On Plant Response To Cadmium, 59(8), 2008, 2205-2219.

[25]. Emamverdian, A., Y. Ding, F. Mokhberdoran, and Y. Xie. Heavy Metal Stresss And Some Mechanism Of Plant Defence Response. The Scientific World Journal, 2015, 1-18.

[26]. Ramel, F., S. Birtic, S. Cuine, C. Triantaphylides, J. Ravanat, and M. Havaux. Chemical Quenching Of Singlet Oxygen By Carotenoids In Plants. Plant Physiology, 158, 2012, 1267-1278.