Macrothink Institute™

Aquatic Science and Technology
ISSN 2168-9148
2014, Vol. 2, No. 2

Detection of Phytochelatin and Glutathione in Seagrass *Thalassia hemprichii* as a Detoxification Mechanism Due to Lead Heavy Metal Exposure

Charlotha I. Tupan

Faculty of Fishery and Marine Science, Brawijaya University, Malang, East Java, Indonesia

Fishery and Marine Science Faculty, Pattimura University, Maluku, Indonesia

E. Y. Herawati

Faculty of Fishery and Marine Science, Brawijaya University, Malang, East Java, Indonesia

D. Arfiati

Faculty of Fishery and Marine Science, Brawijaya University, Malang, East Java, Indonesia

Aulanni'am

Faculty of Mathematics and Natural Science, Brawijaya University, Malang, East Java, Indonesia

Received: April 11, 2014 Accepted: May 15, 2014 Published: July 1, 2014

doi:10.5296/ast.v2i2.5853 URL: http://dx.doi.org/10.5296/ast.v2i2.5853

Abstract

Seagrass *Thalassia hemprichii* is used to study lead (Pb) metal accumulation and synthesis of phytochelatin (PC) and glutathione (GSH) as defense mechanisms against lead toxicity. The plants were exposed by lead (Pb $(NO_3)_2$) metal in some concentrations (0, 5, 15 and 25 ppm) for some periods of time (1, 2, 3 and 4 weeks). The contents of lead (Pb), phytochelatin (PC) and glutathione (GSH) are analyzed in leaf and root tissue. Lead accumulation in seagrass depends on the greatness of metal concentration and length of exposure time where the root accumulates lead higher than the leaf. Glutathione is produced higher in the root during lead exposure, while phytochelatin is produced in week 1 and 2. Then, production of phytochelatin turns to higher in the leaf in week 3, although it decreases in week 4.

Phytochelatin and glutathione are formed at different retention time in UPLC, when glutathione content is formed earlier and lower than phytochelatin. The study not only reveals that content formation of phytochelatin (PC) and glutathione (GSH) occurs to respond Pb metal, but also shows that production of PC and GSH correlates significantly to Pb metal accumulation in the root and leaf of *T. hemprichii*. Therefore, in short, formation of PC and GSH, for sure, is a part of defense mechanisms conducted by seagrass *T. hemprichii* to protect itself against Pb metal toxicity.

Keywords: PC, GSH, Pb, *T. hemprichii*

1. Introduction

Heavy metal becomes one of threats to living organisms since its use raises in various industrial activities and farmlands resulting in high level of bioaccumulation and toxicity. A few recent years, awareness on this matter increases about how heavy metal plays a role as a pollutant to environment (Baker & Walker, 1989), along with its effect to the plants (Cobbet, 2000; Cobbet & Goldsbrought, 2002; Clemens, 2006). Lead (Pb) is one of heavy metals which become a dangerous metal for human and it may destruct environment (ATSDR, 1992). It is known that this heavy metal may become toxicity damaging respiratory system, nervous system and be poison to human blood. For plants, lead impacts on morphology, growth, and photosynthesis process (Yadav, 2010). Besides, high concentrate level of lead may hamper enzyme activity, water imbalance, membrane permeability changes, and may interfere mineral absorption (Sharma & Dubey, 2005). Further, lead may induce oxidative stress due to an increase of reactive oxygen species (ROS) (Reddy *et al.*, 2005).

Heavy metal tolerance and detoxification molecularly to plants may be conducted by a number of different mechanisms, for example pumping-out metal from plasma membrane, producing metal-fastening compound, conducting deposition metal into vacuole (Baycu, 2002; Yadav, 2010). Examined mechanism is the best way conducted by the plants in executing tolerance against heavy metal, it is by synthesizing metal-fastening peptide i.e. phytochelatin (Ahner *et al*., 1997: Baycu, 2002; Gomez *et al*., 2009; Ross, 1994). Phytochelatin is cysteine-rich peptide, capable of fastening metal through SH group in cysteine section, in relation structurally to glutathione, also, it has common structure of (ɣ-Glu-Cys)n-Gly, with various n ranging from 2-11 (Grill *et al*., 1985), yet, in general, it ranges from 2-5 (Cobett, 2000) and depends on kinds, types and level of metal exposure (Grill *et al*., 1985; Rauser, 1990; Cobett & Goldsbrough, 2002).

Phytochelatin will form complexes with metal ion if it is hit by toxic heavy metal, and prevent it from being mixed in cellular metabolism. Then, these complexes are stored in vacuole, so it would decrease adverse effect from the metal ion (Salt & Rauser, 1995). Production of phytochelatin may be used as biochemistry to find out metal toxicity in biota (Ahner *et al*., 1997; Wang *et al*., 2009; Diana *et al*., 2010; Morelli *et al*., 2009). Other than phytochelatin, glutathione is also important for plants. Glutathione is involved in the defense against reactive oxygen species (ROS) (Foyer & Noctor, 2005), heavy metal absorption (Cobbett & Goldsbrough, 2002) and xenobiotic detoxification (Dixon *et al*., 1998). Glutathione (GSH) is sulfur containing thioltripeptide on a formula of ɣ-Glu-Cys-Gly (Cobbett, 2000; Yadav, 2010).

Lots of studies analyzing involvement of phytochelatin and glutathione in metal detoxification were conducted responding to cadmium (Cd) metal, while case of lead (Pb) metal detoxification on plants has not much conducted. Seagrass belongs to sea plants possessing high capacity in absorbing metal due to its direct interaction with water column (through the leaf) and sediment (through the root), so its leaf and root are well-ion absorbing section (Romero *et al*., 2006; Ralph *et al*., 2006). *T. hemprichii* belongs to seagrass species found in tidal flat, scattered all over Indonesian waters (Kiswara & Winardi, 1994) including Ambon Island (Tuhumury, 2008; Tupan 2012). With regards to seagrass ability in absorbing metal, further study is needed regarding plants' mechanisms of defense against metal toxicity, especially lead (Pb). The study aims to analyze lead (Pb) heavy metal accumulation in leaf and root tissue of sea grass *T. hemprichii*, and to analyze production of phytochelatin (PC) and glutathione (GSH) triggered by the existence of lead heavy metal as a part of

mechanisms of detoxification.

2. Materials and Methods

2.1 Lead Exposure

Sample of *T. hemprichii* is taken from Inner Ambon Bay, Ambon Island, Maluku having south latitude of $3^0$38'30" – $3^0$39'30" and east longitude of $128^0$11'30" – $128^0$12'00". Utilized sample of *T. hemprichii* is sample having 3-4 leaves respectively in 3.0-4.5 mm in width and 50-80 mm in length other than healthy leaves. Sample in amount of 30 standing plants are taken care in 48 aquariums with 40 cm in lenght, 40 in width, and 30 cm in height for one week, while acclimatisation given to the sample in dark and ligth periods is 14:10 hours. $Pb(NO_3)_2$ is used for lead exposure to the seagrass in concentration of 0, 5, 15 and 25 ppm by three repetitions for every treatment. Observation is conducted in week 1, week 2, week 3 and week 4. Parameter of water quality remains constant between treatment of control and Pb exposure. Salinity value is ranging from 32.0-33.5 ppt, temperature 27.5 – 29.0^{0}C, pH 7.72 – 8.12 and dissolved oxygen 5.69 – 5.88 ppm; then, it seems that this water quality parameter does not affect stress response at all during the research.

2.2 Lead Accumulation

Seagrass tissue is re-washed by sea water to cleanse sediment and other dirt before conducting further analysis. Cleansing by distilled water may cause premature release of metal and other cations (Ledent *et al*., 1995). Stuck organisms on its surface are also cleaned. Root and leaf tissue are dried up first at room temperature to obtain constant weight, then they are dried into the oven at 100^{0}C for 5 hours. Next, 1 g in weight of dried and refined sample is heated up and dissolved in 5 ml HNO_3 5M. Further, sample is strained, added by distilled water and then analyzed by means of atomic absorption spectrophotometry (AAS) type Shimadzu AA-6200 at wave length of 283,3 nm.

2.3 Determination on the Content of Phytochelatin and Glutathione

Extraction method to phytochelatin and glutathione is conducted by Maier *et al*., (2003) at some modifications. Two gram of every root and leaf of *T. hemprichii* are soaked in 10 mL MSA 10 mM, heated up in water bath at temperature 70^{0}C for 2 minutes and then refined in ice bath for 2-4 minutes. Extracted material, then, is centrifuged for 50 minutes at velocity of 4000 rpm at 4^{0}C. Supernatant is strained and added by MSA 10 mM to reach 20 mL in volume. According to Lima *et al*., (2006), supernatant is derivatized by 62 µL NaOH 0.1 M, 200 µL tris-buffered solution HCL 0.1 M, 63 µL EDTA, 25 µL mercaptoethanol and 50 µL monobromobimane (mBBr) and incubated for 40 minutes at temperature 35^{0}C in the dark room. Next, reaction is stopped by adding acid acetate 5% (v/v) of 1 mL. So, sample is ready to be calculated by means of UPLC (*Ultra Performance Liquid Chromatography*). To identify peaks of formed peptides, researcher applies applicable standard of glutathione (GSH) and phytochelatin (PC2).

2.4 Data Analysis

Data is analyzed by ANOVA multifactorial analysis ($p<0.05$) and Pearson correlation by using GenStat version 14. Data is presented as an average of three repetitions.

3. Results

3.1 Pb Accumulation in the Root and Leaf of Thalassia hemprichii

Seagrass *T. hemprichii* accumulates Pb metal unequally depending on exposure concentration and time and regarding to the analyzed plant tissue. Pb content in root and leaf during exposure increases along with the increase in the treatment concentration (Figure 1). It proves that leaf and root of *T. hemprichii* is able to absorb Pb metal from water and sediment. In the root, Pb accumulation incisively increases from week 1 to week 4 in all treatments, while the highest level occurs in treatment of week 4 in concentration of 25 ppm by 4.067±0.072 ppm. In leaves, increase of Pb accumulation in week 4 is not as high as previous weeks, however, its highest peak also occurs in week 4 in concentration treatment of 2 ppm by 1.623±0.062 ppm. Statistically, it is found that concentration treatment of Pb and exposure time significant effect average of Pb content either in the leaf or root by reaching < 0.001 ($\alpha=0.05$), where the highest accumulation occurs in concentration treatment of 25 ppm in week 4. Leaf and root tissue of *T. hemprichii* shows significant difference in accumulating Pb metal by < 0.001 ($\alpha=0.05$), where the average of Pb accumulation is greater in the root than the leaf.

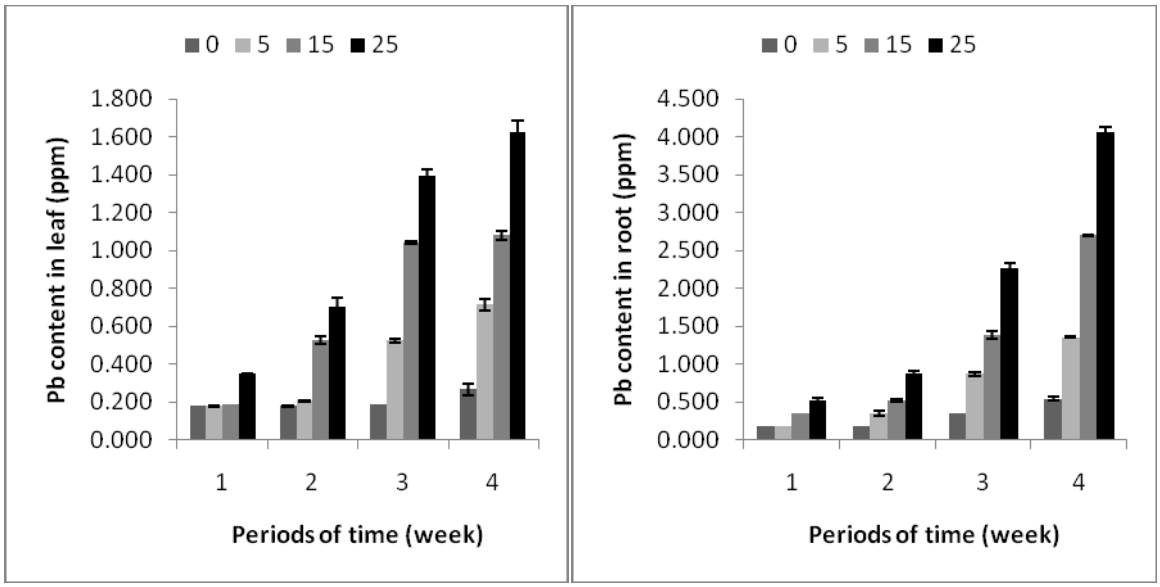

Figure 1. Average of lead (Pb) accumulation in leaf and root tissue of *T. hemprichii*

3.2 Phytochelatin and Glutathione Content in Thalassia hemprichii

Phytochelatin (PC) and glutathione (GSH) were formed by different content in leaf and root tissue of *T. hemprichii* depending on concentration treatment and Pb metal exposure time (Figure 2 and 3). Phytochelatin treatment increases along with the incrase of Pb metal accumulation in leaf and root for all Pb concentration treatments, and reaches its peak in the third week (for concentration of 5, 15, and 25 ppm), and it decreases in the fourth week, while in the controlled treatment, phytochelatin keep increasing up to the fourth week (Figure 2). The highest phytochelatin content in leaf is reached in week 3 at Pb concentration treatment of 15 ppm by 8.571±0.246 ppm, so does the highest content in root, but, it is in different concentration (25 ppm) by reaching 2.358±0.251 ppm (Figure 4).

Glutathione content increases during Pb metal exposure from week 1 to week 4 in all Pb concentration treatment either in leaf or root tissue of *T. hemprichii* (Figure 3). It is different from descending-phytochelatin content in week 4. The highest glutathione content in leaf and root is reached in week 4 in concentration of 25 ppm with value of 2.353±0.203 ppm (leaf) and 2.458±0.215 ppm (root). Average glutathione content in leaf and root tissue is lower than phytochelatin content. Pearson correlation analysis is applied to determine if there is relationship between Pb accumulation in leaf and root of *T. hemprichii* exposed by Pb metal corresponding to its ability in producing phytochelatin and glutathione. Pb content in leaf correlates significantly to phytochelatin content by 0.4822 with p-value by < 0.001 (α=0.05), when glutathione is 0.7295 p-value reaches < 0.001 (α=0.05); phytochelatin content correlates significantly to glutathione content in leaves by 0.4403 and p-value by < 0.001 (α=0.05). In root, Pb content correlates significantly to phytochelatin by 0.3688 with p-value by 0.0027 (α=0.05), when glutathione is 0.7403 p-value reaches < 0.001 (α=0.05); also, phytochelatin content correlates significantly to glutathione content by 0.5492 with p-value by < 0.001 (α=0.05).

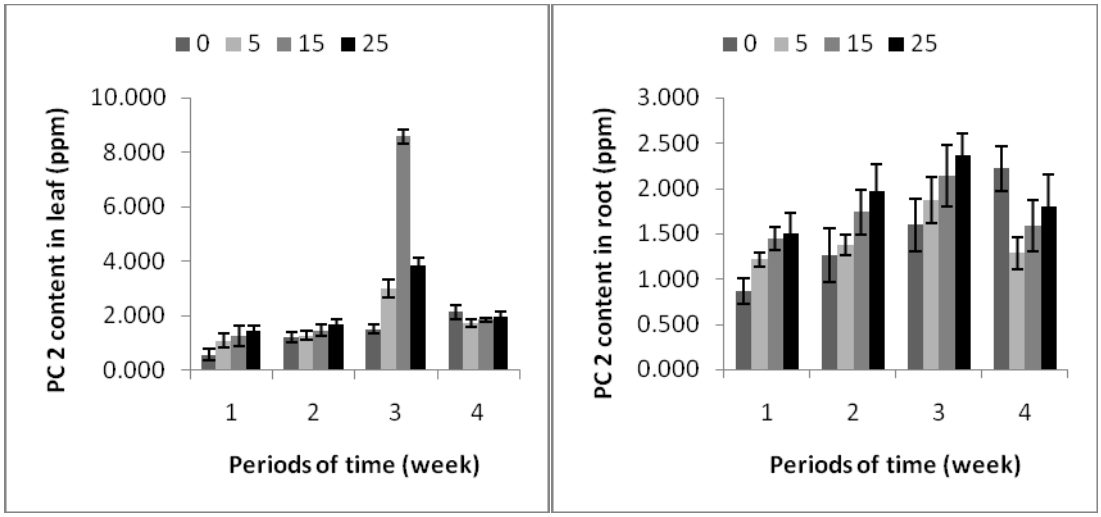

Figure 2. Phytochelatin content in leaf and root tissue of *T. hemprichii*

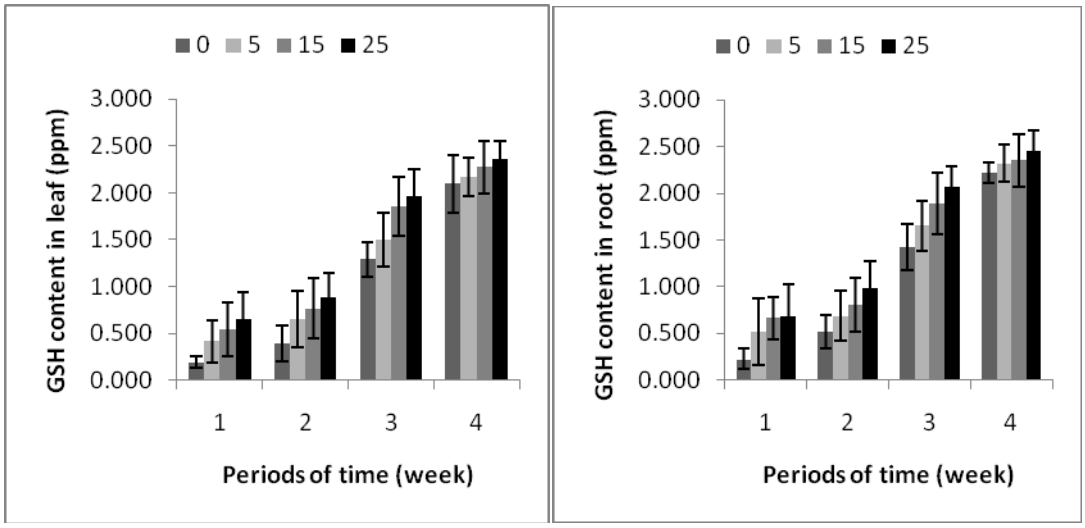

Figure 3. Glutathione content in leaf and root tissue of *T. hemprichii*

4. Discussion

4.1 Lead (Pb) Accumulation in Leaf and Root Tissue of T. hemprichii.

Pb metal in leaf and root of *T. hemprichii* shows that this aquatic plant is able to accumulate metal from water column through the leaves and sediment through the root (Romero *et al*., 2006; Ralph *et al*., 2006). In this study, root has a greater capacity in absorbing and accumulating metal than the leaves. Plant root may absorb heavy metal in form of dissolved ion such as nutritive substances absorbed along with the water, where the root cells, in general, contains higher ion concentration than surrounding medium (Lakitan, 2007), so water diffusion containing Pb metal may occur. Other process backing up Pb accumulation in the root is caused by presence of root absorbing ability in absorbing water and ion in sediment. It is supported by Guilizzoni (1991) that aquatic angiospermae absorbs nutritive substances and heavy metal especially sediment through root hair, then they are translocated to upper plant section since most of aquatic species possess well-developing transportation system where water stream may transport ion through xylem and floem either basipetally or acropetally Marin-Guirao*et al*., (2005) also found that Pb metal concentration in seagrass root of *Comodocea nodosa* is higher than in leaf, it is also explained that leaf of *C. nodosa* will absorb Pb metal from water column when availability of Pb metal in the sediment is low. In this study, lead accumulation is higher when it occurs through sediment by the root since in the beginning, sediment has higher Pb concentration than water column (data is not presented), so it allows greater absorption by the root, then Pb translocation is also occurred basipetally from leaves to root.

4.2 Phytochelatin and Glutathione Content in Thalassia hemprichii

Seagrass *T. hemprichii* produces higher phytochelatin in the root at Pb metal exposure for the first two week. It is endorsed by study of Gomez *et al*. (2009) that an increase in phytochelatin concentration is the response against Pb metal accumulation in plant root of *Salvinia minima*. Some land plants exposed by cadmium (Cd) metal also shows phytochelatin production especially which is coming out of root tissue. It might be possible since cadmium, in fact, is absorbed more into the land than waters (Baycu, 2002). Absorbed metal either into the root or leaves will form complex metal transport which will penetrate xylem and floem vascular tissue and then transported to the leaves and root. One of compounds forming complex with metal is phytochelatin (Salt & Rauser, 1995), therefore, phytochelatin is found in the root and leaf which were accumulated by metal. Mendoza-Cosat *et al*., (2008) found that content of phytochelating and Cd metal is high, so does ratio of PC/Cd and GSH/Cd in floem tissue compared to xylem in *Brassica napus* plant. Then, it may be concluded that translocation is conducted by floem and that PC and GSH functions as Cd transporter for long distance from the leaves to the root.

This study also found that in week 3 and 4, concentration of phytochelatin turns higher in leaves, especially for concentration treatment of 15 ppm. Mishra *et al*., (2006) found a number of PCs in vascular tissue of *Bacopan monnieri* plant which were exposed by Cd metal. Also, it is reported that high PC concentration in the leaves is caused by PC transportation from root through vascular tissue. The study also indicates the influence of xylem and floem in transporting PC when PC content is high in root, other than triggered by Pb content absorbed highly by root from sediment, there found the role from floem tissue transporting PC from leaves to root. Then, when PC turns to high in leaves, PC is not only absorbed from water column by the leaves, but it is also transported from the root through xylem.

Produced phytochelatin in the study has short chain, it is PC2 (homo-phytochelatin) at retention time of 5.68 minutes, and perhaps, PC3 occurs at retention time of 6.26 minutes (Figure 4). According to Grill *et al*., (1985), phytochelatin (PC) has common structure of (ɣ-Glu-Cys)n-Gly, with long chain where n varies from 2-11, while Cobbett (2000) believes that in genereal, n varies from 2-5. Thereby, simply put that accumulation of sea grass *T hemprichii* against the given Pb metal concentration may activate PC in short chain. Detected phytochelatin also has low content (ppm equals to µg/mg) compared to Pb (ppm equals to mg/kg). According to Alvares-Legorreta *et al.*, 2008), other than phytochelatin, there occurs other involved mechanism of defense to respond metal toxicity, for example by storing the metal in cell wall. In this study, anatomically, it is found that a part of Pb metal is attached to either exodermis, endodermis cell wall, or cortex cells (data is not presented). Phytochelatin forms complex with metal ion in cytosol, then it is transported into vacuola (Salt & Rauser, 1995). It is indicated that before penetrating into cytosol and vacuola, metal ion has been hampered in cell wall, so formation of phytochelatin is low. Wojcik *et al*., (2005) reports that capacity in attaching heavy metal to plant cell wall is a preventive effort against loss effect by cutting down amount of heavy metal absorbed into cytosol. Garcia-Rios *et al*., (2007) found that complex polysaccharide in cell wall of red algae *Gracilaria cornea* in intracellular manner turns to be good barrier against heavy metal accumulation.

Not only phytochelatin (PC), but also glutathione (GSH) also plays an important role in process of detoxifying xenobiotic and heavy metal, and also in mechanisms of defense against reactive oxygen species (ROS). This glutathione action also plays a role as a pioneer for phytochelatin synthesis (Yadav, 2010). The study shows that glutathione (GSH) peak appears at retention time of 3.38 minutes before the appearance of phytochelatin (PC) peak, but it has a lower concentration than phytochelatin. It indicates that glutathione (GSH) plays a role in phytochelatin (PC) synthesis and it could probably use in restraining ROS by Pb metal. Other study also reports that synthesized phytochelatin after being exposed by Pb metal will decrease GSH content, and if production of PC is too much, it will cause GSH thinning and henceforth, it causes oxidative stress (Mishra *et al*., 2006). According to Mehra and Tripathi (1999) and Sharma and Shanker (2005), metal sequestration by PC and GSH is the best known mechanism in process of detoxifying Pb in plants. This study not only discovers that content formation of phytochelatin (PC) and glutathione (GSH) occurs in response to Pb metal, but also shows that production of PC and GSH correlates significantly to Pb metal accumulation either in the root and leaf of T. hemprichii. Therefore, in case of *T hemprichii* exposed by Pb metal, the study shows that formation of PC and GSH, for sure, belongs to part defense mechanisms of the seagrass to protect itself against Pb metal toxicity.

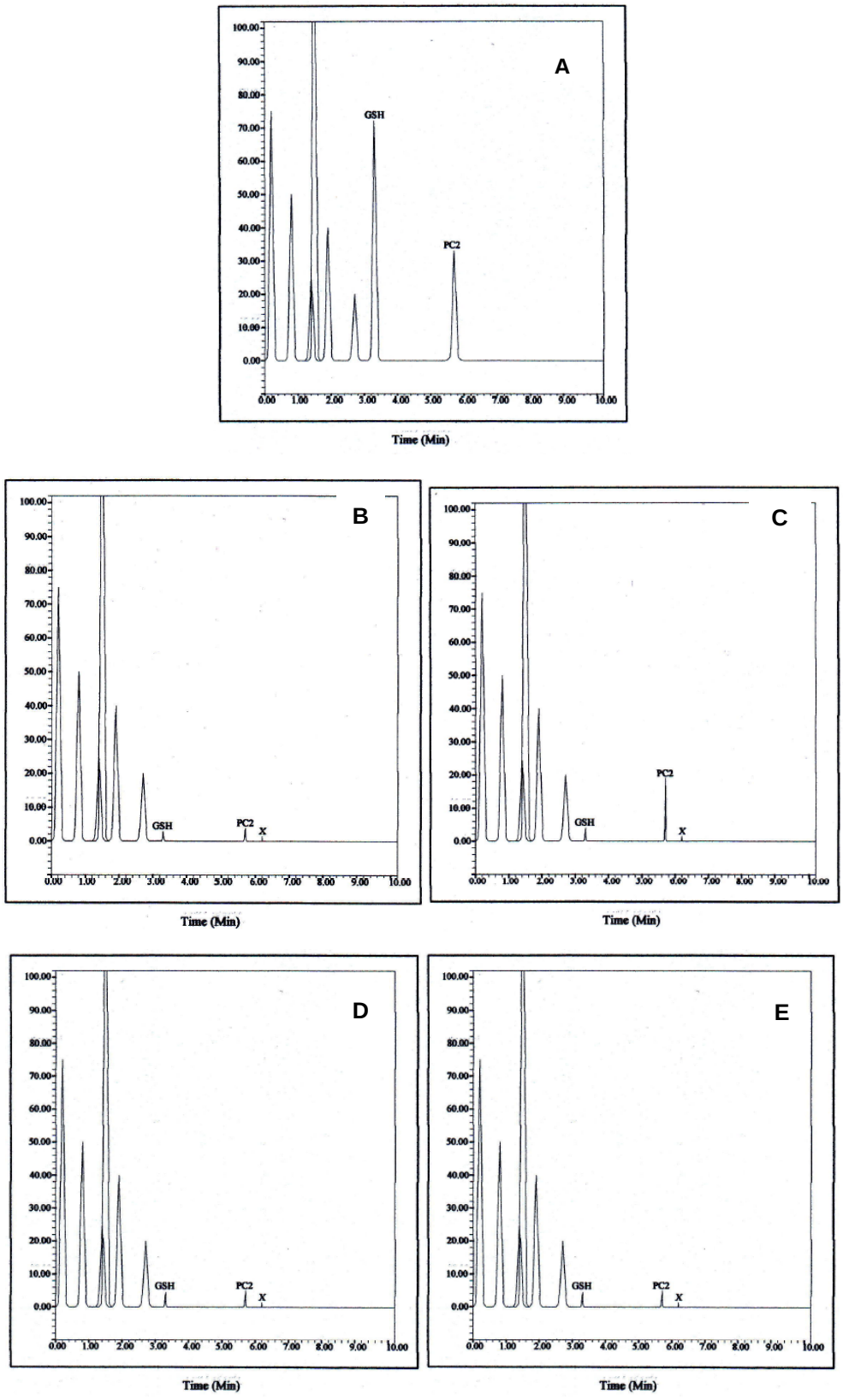

Figure 4. UPLC Profile of PC and GSH in *Thalassiahemprichii* in week 3

Notes: A = Standard; B = leaf control; C = 15 ppm leaf; D = root control; E = 15 ppm root; GSH = Glutathione; PC2 = homo-phytochelatin; X = other peptide (possible PC3)

5. Conclusion

Seagrass *Thalassia hemprichii* accumulates lead metal in all tissues, i.e. in leaves and root where the highest level of lead is accumulated in the root. Lead accumulation occurs in response to metal concentration and exposure time. Exposed lead concentration may produce phytochelatin and glutathione in leaf and root as mechanisms of defense againts toxicity of the metal.

Acknowledgement

My best gratitude to Director Generals of Higher Education, Ministry of Education and Culture of Republic of Indonesia for funding this study and research. Thanks also go to Rector of Pattimura University for allowing me conduct the study and Rector of Brawijaya University for giving me the chance to conduct the study in the University. Also, my best gratitude to the Chief and Staffs of Environment Laboratory, Chemistry Department, School of Mathematics and Physics Brawijaya University for analyzing lead metal, Water Sciences Laboratory, School of Marine and Fisheries Brawijaya University for facilitating us seagrass aquaculture, and Pharmacokinetic Laboratory, School of Medicine Padjadjaran University, Bandung for analyzing phytochelatin and glutathione.

References

Alvares-Legorreta, T., Mendoza-Cozalt, D., Moreno Sanchez, R., Gold Boichot, G. (2008). Thiol Peptides induction in the seagrass Thalassia testudinum (Banks ex Konig) in response to cadmium exposure. *Aquat.Toxicol, 86*, 12-19. http://dx.doi.org/10.1016/j.aquatox.2007.09.001

ATSDR. (1992). Toxicologycal Profile for Lead. Agency for Toxic Substances and Desease Registry. Atlanta, GA, USA.

Baycu, G. (2002). Phytochelatin Biosynthesis and Cadmium Detoxification. *Cell and Molecular Biology, 1*, 45-55.

Clemens, S. (2006). Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochem., 88*, 1707-1719. http://dx.doi.org/10.1016/j.biochi.2006.07.003

Cobbett, C. (2000). Phytochelatin and their roles in heavy metals detoxification. *Plant Physiol, 123*, 825-832. http://dx.doi.org/10.1104/pp.123.3.825

Cobbett, C., & Golsbrough, P. (2002). Phytochelatin and metallothioneins: roles in heavy metal detoxification and homeostatis. *Annu. Rev. Plant Biol., 53*, 159-182. http://dx.doi.org/10.1146/annurev.arplant.53.100301.135154

Dixon, D. P., Cummins, L., Cole, D. J., & Edward, R. (1998). Glutathione mediated detoxification systems in plant. *Current Opinions in Plant Biology, 1*, 258-266. http://dx.doi.org/10.1016/S1369-5266(98)80114-3

Garcia-Rios, V., Freile-Pelegrin, Y., Robledo, D., Mendoza-Cozalt, D., Moreno-Sanchez, R., & Gold Bouchot, G, G. (2007). Cell wall composition affects Cd accumulation and intracellular thiol peptides in marine red algae. *Aquat.Toxicol., 81*, 65-72. http://dx.doi.org/10.1016/j.aquatox.2006.11.001

Gomez, N. E., Cozatl., D. M., Sanches., R. M., Mendoa., D. G., Perez., O. Z., Hernandez, A. M., & Santamaria, J. M. (2009). The PbHyperaccumulator aquatic fern *Salvina minima* (Baker), Responds to Pb^{2+}By Increasing Phytochelatins Via Changes in SmPCS Expression

and in Phytochelatin Synthase Activity. *Aquatic Toxicology, 91,* 320-328. http://dx.doi.org/10.1016/j.aquatox.2008.11.002

Grill, E., Winnacker, E. L., & Zenk, M. H. (1985). Phytochelatin: the principal heavy metal complexing peptides of plants. *Science, 230*, 474-676. http://dx.doi.org/10.1126/science.230.4726.674

Guilizzoni, P. (1991). The role of heavy metals and toxic materials in the physiological ecology of submersed macrophytes. *Aquat. Bot., 41*, 87-109. http://dx.doi.org/10.1126/science.230.4726.674

Kiswara, W., & dan Winardi. (1994). Keanekaragaman dan sebaranlamun di Teluk Kuta dan Teluk Gerupuk, Lombok Selatan. *Dalam*: *Struktur komunitas biologi padang lamun di pantai selatan Lombok dan kondisi lingkungannya.*

Lakitan, B. (2007). *Dasar-dasar Fisiologi Tumbuhan*. Jakarta: PT Raja Grafindo Persada.

Ledent, G., Mateo, M. A., Warnau, M., Temara, A., Romero, J., & Dubbois, Ph. (1995). Element losses following distilled water rinsing of leaves of the seagrass Posidoniaoceanica (L) Delile. *Aquat. Bot., 52*, 229-235. http://dx.doi.org/10.1016/0304-3770(95)00506-4

Lima, A. I. G., Pereira, S. I. A. P., Figuiera, E. M. A. P., Cladeira, G. C. N., & Caldeira, H. D. S. Q. (2006). Cadmium detoxification in roots of Pisumsativum seedlings: relationship between toxicity levels, thiol pool alterations and growth. *Environ Exp Bot*., *55*, 149-162. http://dx.doi.org/10.1016/j.envexpbot.2004.10.008

Maier, E. A., Matthews, R. D., Mc Dowell, J. A., Walden, R. R., & Ahnes, B. A. (2003). Environmental cadmium level increase phytochelatin and Glutathione in lettuce grown in a chelator-bufferd nutrient solution. *Environ. Qual*., *32*, 1356-1364. http://dx.doi.org/10.2134/jeq2003.1356

Marin-Guirao, L., Marin, A., Lioret, J., Martinez, E., & Garcia, A. J. (2005). Effect of minning wastes on a seagrass ecosystem: metal acumulation and bioavilabillity, seagrassdinamics and associated community structure. *Mar. Environ. Res*., *60*, 317-367. http://dx.doi.org/10.1016/j.marenvres.2004.11.002

Mehra, R., & Tripathi, R. (1999). Phytochelatin and tolerance. In Agralwal, S. B., & Agrawal, M. (Eds.), *Environmental pollution and Plant Responses* (pp. 367-382). CRC Pres, Lewis Publisher, Boca Raton, FL.

Mendoza-Cozalt, D. G., Butko, E., Springer, F., Torpey, J. W., Komives, E. A., Kehr, J., & Schroeder, J. L. (2008). Identification of high levels of phytochelatins, glutathione and cadmium in the phloem sap of Brassica napus. A role for thiol peptides in the long distance transport of cadmium and the effect of cadmium on iron translocation. *Plant J*., *54*, 249-259. http://dx.doi.org/10.1111/j.1365-313X.2008.03410.x

Misra, S., Srivastava, S., Tripathi, R., Kumar, R., Seth, C., & Gupta, D. (2006). Lead detoxification by contail (Ceratophyllum demersum L) involves induction of phytochelatins and antioxidant system in response to its accumulation. *Chemosphere*, *65*, 1027-1039. http://dx.doi.org/10.1016/j.chemosphere.2006.03.033

Ralph, P. J., Tomasco, D., Moore, K., Seddon, S., & Machinnis-Ng, C. M. O. (2006). Human impact on seagrasses: eutrophication sedimentation and contamination. In Larkum, A. W. D., Orth, R. J., & Duarte, C. (Eds.), *Seagrasses: Biology, Ecology and Conservation* (pp.

567-593). Springer.

Rauser, W. E. (1990). Phytochelatin. *Annu. Rev. Biochem.*, *59*, 61-86. http://dx.doi.org/10.1146/annurev.bi.59.070190.000425

Reddy, A. M., Kumar, S. G., Jyonthsnakumari, G., Thimmanaik, S., & Sudhakar, C. (2005). Lead induce changes in antioxidant metabolism of horsegram (Macrotylomauni florum (Lam.) Verdc) and bengalgram (Cicerarietum L). *Chemosphere, 60*, 97-104. http://dx.doi.org/10.1016/j.chemosphere.2004.11.092

Romero, J., Alcoverro, T., Crego, B. M., & Peres, M. (2006). The Seagrass Posidonia oceanica as a Quality Element Under The water Framework Directive: POMI, a Multivariate Method to Assess Ecological Status of Catalan Coastal Waters. *Working document of the POMI group, University of Barcelona and Centre d'EstudisAvancats de Blanes (CSIC).*, 15 pp.

Salt, D. E., & Rauser, W. E. (1995). MgSTP-dependent transport of phytochelatins across the tonoplast of the roots. *Plant Physiology*, *107*, 1293-1301.

Scarano, G., & Moreli, E. (2002). Characterization of Cadmium and lead phytochelatin complexes formed in a marine microalga in response to metal exposure. *Biometals.*, *15*, 145-151. http://dx.doi.org/10.1023/A:1015288000218

Sharma, P., & Dubey, R. S. (2005). Lead toxicity in plant. *Brazilian Journal of Plant Physiology, 17*, 35-52. http://dx.doi.org/10.1590/S1677-04202005000100004

Tuhumury, S. F. (2008). Status KomunitasLamun di PerairanPantaiTeluk Ambon Dalam.*Ichthyos*. *Jurnal Penelitian Ilmu-Ilmu Perikanandan Kelautan*, *7*(2), 85-88.

Tupan, Ch. I. (2012). Status Komunitas Lamun di Perairan Pantai Rutong, Bagian Selatan Pulau Ambon, Maluku. Proceeding SENTA.Fak.Teknologi Kelautan. ITS. Surabaya.

Wojcik, M., Vangronsveld, J., D'Haen, J., & Tukiendorf, A. (2005). Cadmium tolerance in Thlaspicaerulescens. *Environmental of Experimental Botany*, *53*, 163-171. http://dx.doi.org/10.1016/S0098-8472(04)00047-4

Yadav, S. K. (2010). Heavy Metals Toxicity in Plants: An Overview on The Role of Glutathione and Phytochelatines in Heavy Metal Stress Tolerance of Plant. Review. *South African Journal of Botany, 76*, 167-179. http://dx.doi.org/10.1016/j.sajb.2009.10.007

Copyright Disclaimer