Research Inventy: International Journal Of Engineering And Science
Vol.3, Issue 3 (June2013), PP 06-12
Issn (e): 2278-4721, Issn (p):2319-6483, Www.Researchinventy.Com

Activation, Concentration, and Expression of Metallothionein-1 On Sea Urchin as Biomonitoring Heavy Metal Cadmium

[1]Dominggus Rumahlatu, [2]Aloysius Duran Corebima, [2]Mohamad Amin, [2]Fatchur Rohman

[1]*Department of Biology, Faculty of Teacher Training and Education Science, Pattimura University, Indonesia.*
[2]*Department of Biology, Faculty Mathematics and Science, State University of Malang, Indonesia*

ABSTRACT *- Molecular techniques such as immunohistochemistry, ELISA and western blotting is highly beneficial to determine the existence of an environmental stressor, such as cadmium (Cd) heavy metal by looking at its activation, concentration and expression toward Metallothionein-1 (MT-1) protein. In this study, conducted experiment for 4 weeks, in order to investigate effect of Cd heavy metal treatment toward MT-1 protein of sea urchin Deadema setosum. This study was conducted in LIPI Ambon, Indonesia laboratory in 100 × 60 × 70 cm^3 aquarium 6 tank. Each tank was filled with 200L of seawater that being changed once a week. Concentration of treatment were 0.0, 1.0, 3.0, 6.0, 9.0, and 12.0 µg/L Cd solution. Each tank was filled with 7 individual of D. setosum as 7 replication. MT-1 activation examination was done in liver organ of D. setosum using immunohistochemistry, MT-1 concentration measurement was done using ELISA, while MT-1 expression examination was done using western blotting at Physiology Laboratory of Medical Faculty of Brawijaya University, Indonesia. Data concerned with concentration of MT-1 was analyzed using One Way Anova and advance test was one using 0.05 Duncan Test. Result of this study showed that Cd treatment is highly significant in increasing concentration of MT-1; higher Cd content would resulted higher concentration of MT-1. Concentration of MT-1 in concentration 12 µg/L is the highest compared to control. Histological view of liver organ of D. setosum experiencing MT-1 protein activation based with immunohistochemistry is highly appropriate with each recorded concentration of of MT-1 protein. This results showed that MT-1 has the potential as seawater pollution biomonitoring of Cd heavy metal pollution viewed from D. setosum biomolecular level.*

KEYWORDS *- Cadmium, Deadema setosum, Metallothionein-1, Pollution Biomonitoring*

I. INTRODUCTION

Heavy metal is a natural component in the earth surface. It has entered the ecosystem waters through the coastal areas which is flown from rivers and coastal abrasion as the result of tide activity and food chain in geochemistry process [1-3]. One of high toxicity heavy metal is cadmium [1]. Compared to other kinds of heavy metal, cadmium (Cd) has high toxicity for animals and plants [4], wide distribution, and long spare time (biological life) in the organism body about 10-30 years because it couldn't be reducted [2,3,5-13], and can effect on serious health problems if it was absorbed in the human's body [14]. Otherwise, Cd in the coastal areas is a polluted resource from human activity like earth heat industry, building materials industry, mine areal, and metal industry [1,4,15], and it was absorbed by free ions in the coastal areas (Cd2+) [1].

In the high level concentration, Cd is carcinogen heavy metal, mutagenic, and teratogenic for some animals [16]. Sea urchin is known as a coastal biota which is sensitive to metal pollutant [17-19]. *Deadema setosum* is one of sea urchin from Deademetidae family, which has high sensitivity to cadmium heavy metal, and as indicator species from the coastal environment [20-22]. The continuous explanation of *Paracentrotus lividus* embryo and high concentration Cd made some abnormality like late development, decrease of stomach length, and bone defect [18]. Cd heavy metal can make eating and development patterns of *D. setosum* [23]. On the other hands, biomarker in the shells like glutathione (GSH) and metallothionein (MT) *Paracentrotus lividus* also used to evaluate the contamination of Cd heavy metal on the coast [18,24]. Explanation of Cd heavy metal with high concentration made late development of *Paracentrotus lividusis* embryo and biomolecular response development of expression protein HSP60 and HSP70 [25]. Cd can also trigger histopathology change and lipid peroxide in liver and roden kidney [26].

In general, every organism has tolerant to heavy metal especially nonessential heavy metal and to control concentration of essential heavy metal in a cell [26,27]. Tolerant of organism to the heavy metal contamination depends on gens of molecules that have roles to do detoxification of intracellular heavy metal. Some types of identified molecules did also detoxification, but many researchers concern with protein MT [28]. MT was included in protein family with relative molecule mass which consists of 4 protein classes, and multi isoform per class [29]. Four isoform protein MT have different distribution, where MT-1 and MT-2 have almost all body tissue, MT-3 can be expressed in brain, and MT-4 can be found in squamosa layer [30].

Some researches identified the expression of protein MT after heavy metal induction on *Lubricus rubellus.* Since then, protein MT has two main functions that is, heavy metal detoxification and free radical scavenger [31-33]. Most researches studied about potential of protein MT as biomarker or biologic signer to existence of heavy metal types in the environment [34]. Then, the research concerned to find out Cd heavy metal detoxification mechanisms with protein MT by using animals [11] Therefore, this research was done in order to reveal Cd heavy metal behavior effect to protein MT-1 on liver *Deadema setosum* as a biomonitoring alternative of coast pollution by Cd heavy metal in molecular level.

II. MATERIALS AND METHODS

This research is done by experiment for 4 week. The tested biota in this research is *D. setosum* sea urchin from breeding result for 1 year in Laboratory UPT Conservation of Sea Biota Office, LIPI in Ambon, Indonesia. *Deadema setosum* is 90 g weight, circle or body diameter 15 cm, and there are 20 kinds.

D. setosum is categorized into 4 levels Cd Laboratory UPT Conservation of Sea Biota Office, LIPI in Ambon, Indonesia concentration in aquarium tanks (1 control tank and 5 tanks to explain $CdCl_2$ concentration), and there are 7 kinds in each group so that the total of analysis unit is 42. Treatment to explain Cd heavy metal is conducted in 6 aquarium tanks of 100 × 60 × 70 cm, where the five tanks were put on the wooden table and were filled with sea water 200 L. Next is the categorized *D. setosum* was poured into each aquarium tank which was consisted of 7 kinds for adaptation phase sake for one week. After that, treatment to heavy metal concentration behavior is done successively 0.0, 1.0, 3.0, 6.0, 9.0, and 12.0 µg/L Cd which was dissolved with air circulation in the aquarium tanks by using aerator electric.

During the treatment, a measurement of physic chemistry factor was taken with the temperature (26-30 ^oC), pH (7.5-8.5 ppm), salinity (15-20‰), oxygen dissolved (5 mg/L) as control variable to change water treatment if the treatment of physic chemistry factor water changes from its highest and lowest limit for sea urchin persistence sake in natural condition. seagrass as feed of *D. setosum* is 30 g/ind/day in the morning by tied seagrass up to a slab of coral, then it was put into treatment tanks and to spread the seagrass on the surface. While, treatment of sea water and feed to the seagrass in explaining Cd heavy metal was begun with pre test to amount of Cd in sea water and feed seagrass by using Atomic Absorbsion Spectrofotometer (AAS), where amount of Cd heavy metal is 0.001 ppm.

After 4 weeks treatment, a surgery to 42 kinds *D. setosum* was done to take liver organ. The liver organ was put into sample vase to examine the activation of protein MT-1, to measure the concentration of protein MT-1, and to examine the expression of protein MT-1 in Medical Physiology Laboratory at Brawijaya University, Indonesia.

2.1. Examine Activation of Protein MT-1 with Imunohistochemistry Method (IHC)

Examination of protein MT-1 on liver *D. setosum* was begun by making liver specimen preparat through tissue fixation phase, making paraffin block, and cutting paraffin block. After the slide of examine activation protein MT-1 is available, then soak the tissue slide with xilena twice, which was 15 minutes per each. Next, it was incubated in ethanol 100% I, 100% II, 95%, 90%, 80%, 70% for 5 minutes per solution. Then, it was incubated again in water for 5 minutes. After that, the available slide was soaked in H_2O_2 0,3% for 30 minutes room temperature. The frozen part, the incubation time was 10-30 minutes, while the frozen tissue array was 5-10 minutes. The slide was rinsed off with water and followed by 1 x PBS (Sigma) (pH 7.4) once, and the circle in the tissue with Pap Pen. It was incubated with 1% normal serum/PBS [Mix 1 x 3,5 ml PBS, pH 7,4 for 1 drop (around 35 µl/drop) normal serum in a tanke for 30 minutes room temperature. It used also PBS which was diluted with antibody in damp room for 1 hour room temperature. It would be rinsed off again with 1 x PBS three times for 5 minutes. It was incubated by diluting the PBS Biotin-label secunder antibody for 30 minutes room temperature. It was rinsed off with 1 x PBS three times for 5 minutes. Then, to prepare detection liquid: Mix 1 x PBS 1,33 ml, 1 drop (around 35 µ/drop) from solution A and 1 drop (around 35 µ/drop) solution B in a tanke, and to incubate the mixed process for 30 room temperature. After that, put the detection liquid in the tissue and incubated it for 30 minutes room temperature. The slide was rinsed off with 1 x PBS three times which was 5 minutes each time. It was give DAB (Diamino-benzedine tetrahydrocloride). The other cells were dropped by counterstains and hematoxilen for 10 minutes. The cell was washed with flowing water, then aquades for 10 minutes. Leave it in room temperature. The tissue was put in the object glass and dropped by entelan. To continue with liver cell which experienced with protein MT-1 activation was observed by using Olympus microscope and blot slide photography with zoom view 400.

2.2. Measurement of Protein MT-1 Concentration with ELISA Indirect Method (Enzyme Linked Imunoassay)

Measurement of protein MT-1 concentration with ELISA indirect method (Enzyme Linked Imunoassay) [35]. Sample liver *D. setosum* organ was grilled with *thawing*. To continue with test with ELISA reader by creating ELISA plate sketch and coating buffer were based on sample code and location of putting sample. Next is doing Coating Antigen with amount 1:40 that was diluted with coating buffer and to be incubated at 4^0 a night. After a day, plate was washed 6 times with PBS Tween liquid 0.2% same as 100ul. Then, we put 100 ul anti body prime anti MT-1 (1:400) in assay buffer. We continued with plate ELISA which was incubated in room temperature for 2 hours and was shaked by plate ELISA shaker. Next step, to wash plate ELISA with TBS Tween liquid 0.2% same as 200ul for 6 times repetition. To add 100ul anti body secunder IGg biotin anti rabbit (1:800) in assay buffer and to incubate while shaker for 1 hour room temperature. To continue with plate was washed again using PBS Tween 0.2% for 6 times. Then, we put 100ul SAHRP liquid (1:800) in assay buffer and to incubate while shaker for 1 hour room temperature. After that, liquid was mixed with PBS Tween 0.2%same as 200ul for 6 times. We added 100ul for each well substrat sure blue TMB microwell, incubate for 20-30 minutes in dark room. In this step, if reaction between antigen and anti body occured, so liquid changed into blue. We continued to add 100ul HCl 1N as stop reaction. So sample was read by using ELISA reader with tide length 450 nm. The absorbant result was conversed with standard curve and we can find out amount of MT-1every sample finally.

2.3. Examine Expression Protein MT-1 With Western Blotting Method

Examine expression MT-1 with western blotting [36]. This examination was preceded by examining SDS-PAGE, that was, electrophoresis sample protein standard broad range (BioLab). Gel of SDS-PAGE was soaked in dionize for 5 minutes. Gel, membrane NC and spon were soaked in transfer buffer for 5 minutes. Next, they were arranged into blackable, spon, 2 pieces of filter paper, gel, membrane NC, 3 pieces of filer paper, spon, whiteable. Then, we put them in chamber which were flown through electricity from negative pole to positive pole (100 volt, 120 minutes). After that, we rinsed off membrane NC with dionize 3 times, soak in Blocking Buffer (BSA 5%), and incubated in overnight temperature 4^0 Celcius. Membrane NC was washed with TPS Tween 0.2% three times of 5 minutes per each, and added anti body in TBS BSA 1%. Next is incubation for 2 hours and shaking. So, gel was washed with TBS Tween 0.2 % for 3 times of 5 minutes per each. In advance, to add Igg biotin and anti rabbit in TBS, we did incubation for 1 hour and shaking. Then, to wash again with TBS Tween 0.2% for 3 times of 5 minutes per each, to add SHARP in TBS, and to incubate for 1 hour and shake them all. After that, to wash with TBS Tween 0.2% for 3 times 5 minutes per each. To add Substrat TMB Membran for 15-30 minutes until a ribbon appears on the membrane. Lastly, to stop the reaction with aquades.

III. DATA ANALYSIS

Data was found through analysis descriptive by using a quantitative method in percentage to describe activation, concentration, and expression protein MT1 in liver *D. setosum.* Besides, we used also the statistic of inferential One Way ANOVA to determine behavior effect of Cd metal to concentration of protein MT-1 and to test Duncan 0.05 to see the average difference of concentration of explanation Cd heavy metal concentration of protein MT-1 in liver *D. setosum.*

IV. RESULT AND DISCUSSION

4.1. Activation, Concentration, and Expression of Protein MT-1

Review result of imonohistochemistry tissue liver *D. setosum* used antibody rabbit anti MT-1 (Fig. 1), which shows that cell has experienced with activation protein MT-1 in brown color, but it has not experienced for the purple one. This result shows that activation of protein MT-1 is developed with high explanation of concentration Cd heavy metal. We can see that cell experienced with activation of protein MT-1 in brown color, spread and form cell groups. This brown morphology cell has reflected activation of protein MT-1 either in cytoplasma and or nucleus in cell liver *D.setosum.* The higher explanation of concentration Cd heavy metal, so brown liver cell indicated the presence of activation protein MT-1 was higher too. Higher concentration of Cd heavy metal resulted cell structure change and related to strategy protective *D. setosum* to fight against stress from accumulation Cd heavy metal. It could be proven from activation protein MT-1 *D. setosum* was high developed with high concentration of Cd heavy metal (Fig. 1).

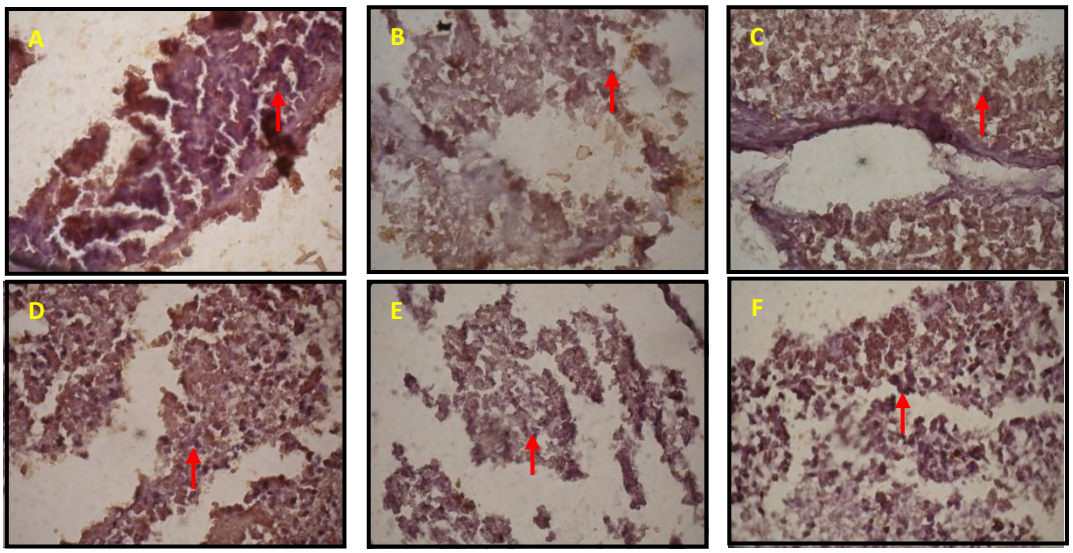

Fig. 1. Review result of imonohistochemistry used antibody rabbit anti MT-1 tissue liver *Deadema setosum.* Picture with notation: A) control; B) concentration 1,0 µg/L Cd; C) concentration 3,0 µg/L Cd; D) concentration 6,0 µg/L Cd; E) concentration 9,0 µg/L Cd; and F) concentration 12,0 µg/L Cd. The arrow signs were used to show liver cell experienced activation protein MT-1 with brown cell.

On the other side, the quantitative result with ELISA test (Fig. 2) showed that the more developed concentration the higher was explanation of Cd heavy metal heavy metal. We can see that the amount of concentration protein MT-1 increased successively from low to high in the tank 1 < 2 < 3 < 4 < 5 < 6. This occurred based on the examination result expression protein MT-1 from *western blotting* test, where protein MT-1 was colored with rabbit anti body anti MTF-1. It was shown by the thicker ribbon protein MT-1 the higher was explanation of Cd heavy metal. Concentration of MT-1 in treatment dozes 12 µg/L Cd was the highest, that was almost 5 times compared to control dozes with the highest expression level. The description of expression protein MT-1 was based on western blotting which suited in every concentration protein MT-1 as recorded on ELISA test.

The development of concentration and expression protein MT-1 related to the higher amount of heavy metal which was accumulated in liver *D.setosum* tissue, so that protein MT-1 was a mental catcher protein. Protein MT-1 did a detoxification to Cd heavy metal that was accumulated on liver *D.setosum.*

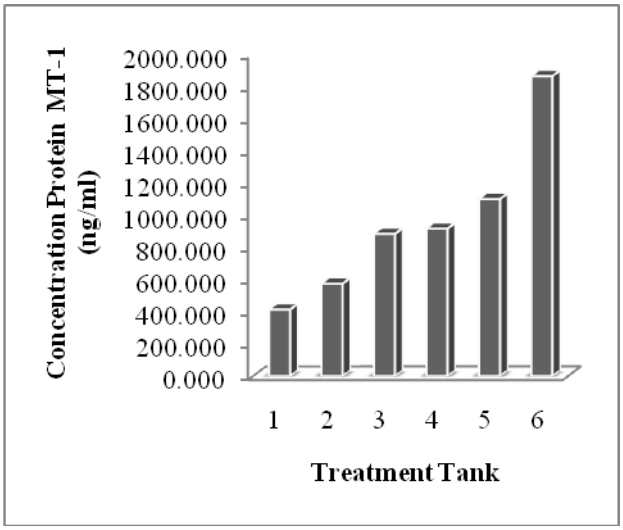

Fig. 2. Concentration protein MT-1 in liver *Deadema setosum* as result of explanation of concentration Cd heavy metal.

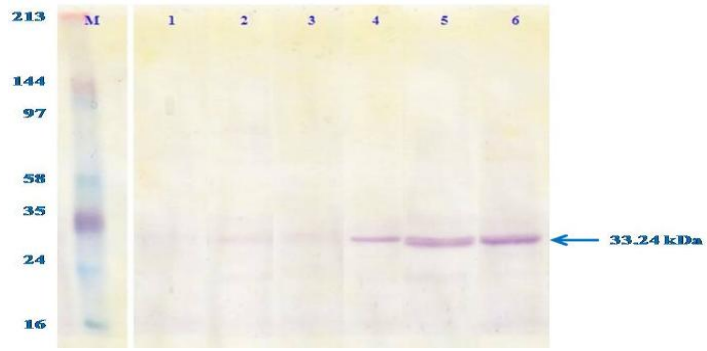

Fig. 3. Expression MT-1 through western blotting test on liver *Deadema setosum* in 6 tanks of concentration Cd heavy metal. Gel of electrophoresis SDS-PAGE was given western blotting test, that was, incubation with anti body monoclonal rabbit anti MT-1 as primer and secondary anti body tank anti rabbit IgG biotin. It was shown on the concentration level in 6 teratment tanks with brown violet color that the protein MT-1 symbolized by arrow. M: Marker, 1 s.d 6: concentration Cd in 6 treatment tanks.

Protein MT-1 gives effect of hampered, stimulation, and homeostasis. The hampered effect of MT-1 related to the neutralizer process and to break ROS, to hamper sitokin pro-inflammation, makrorag, and limfosit T, and to hamper apoptosis (cytochrome c, p53, and caspase). Effect of stimulation MT-1 related to its roles as apoptosis hamper and development factor, and also the increase of functional recovery process. The mechanism of homeostasis MT-1 related to control and regulation of toxic and heavy metal [37]. The increase of accumulation Cd heavy metal in cell would change histopathology and peroxidation lipid in liver and kidney [26]. Cd heavy metal can cause osteoporosis which was deposited in liver and kidney on rat [13]. This supposed correlated with the separation of Cd heavy metal from rotein MT-1 to make accumulation Cd heavy metal and they can affect cell organelles.

In relation to the difference of cell morphology that show activation of protein MT-1 as result of the existence of Cd heavy metal, revealed that protein MT-1 consists of isoform MT-1 and MT-2 on animals which have high sensitivity to existence of heavy metal [29]. Activation of protein MT was inducted by free radical (Reactive Oxygen Species/ROS), glukokorticoid (GC) and some sitokin [38]. Generally, the synthesis control mechanism of protein MT were involving protein Metal Transcription Factor-1 (MTF-1) [32] which was a censor Zn in cytoplasm [39] and has correlation with gens that role in homeostasis Zn process. After the accumulation process, the activated MTF-1 would experience translocation into nucleus and Metal Response Element (MRE) on gen MT promoter in order to trigger the expression of protein itself. [40]

4.2. The Effect of Cd Heavy Metal to Concentration Protein MT-1 in Liver *Deadema setosum*

The result of variant analysis showed that effect of concentration Cd heavy metal was very significant ($p < 0.05$) to concentration protein MT-1 in liver *D. setosum*. This result was in compliance with imonohistochemistry test (Fig. 1), ELISA test (Fig. 2), and western blotting test (Fig. 3) that showed development of activation, concentration, and expression of protein MT-1 parallel to the higher concentration explanation of Cd heavy metal. This result also explained that response of biomolecular *D. setosum* would be activated for the accumulation of Cd heavy metal. Exixtence of protein MT-1 in cell has experienced physiology pressure as related to its function in protecting toxicity of heavy metal and stress oxidative effect [41]. It means that expression protein MT-1 has effected to hamper cell distraction where MT-1 experienced detoxification to Cd heavy metal existence.

On the other hands, the result of Duncan test implied that there were average treatment differences on group of Cd concentration level. It showed that Cd concentration was increased protein MT-1 significantly. The higher concentration Cd was explained the higher was concentration protein MT-1 that activated to *D. setosum*. High activation, concentration and expression of protein MT-1 has connected to resistance mechanism of cells to decrease cell distraction. In other words, we can say that MT-1 stacked to Cd heavy metal or activation, concentration, and expression of MT-1 was effected by Cd heavy metal existence. Protein which constantly lost ability as result of Cd explanation could get its ability back after its incubation with metallothionein (Cd-MT) [42]. Protein MT-1 could develop the functional recovery [37]. Furthermore, protein MT-1 was used to biomarker heavy metal and could also do detoxification heavy metal function and get back the metabolism enzyme function that has inactivated by heavy metal induction [11].

On the other side, the biomolecular response which was result of explanation of Cd heavy metal, was not only limited by activation, concentration and expression of protein MT-1 as shown, but also whether protein MT-1 exixtence on *D. setosum* can be as percentage of polluted environment. When explanation of heavy metal stressed on the environment, many changes will occur on the level of biochemistry, physiology, and histology of live organism. These changes can be used as biomarker to sign the existence of environment stressor [43].

This research result has shown the presence of activation, concentration, and expression of protein MT-1 resulted by accumulation of Cd heavy metal. The question now is whether this result can be used as model of biomonitoring explanation Cd heavy metal on biomolecular level by using *D. setosum* as biomonitoring species? Biomonitoring is an evaluation technique to explanation to chemistry materials based on sampling and tissue analysis, liquid, and kindsal tissue [44]. On the other hands, biomonitoring can be used to measure the amount of chemistry materials, including heavy metal in environment [45].

Data analysis of this research showed that protein MT-1 on *D. setosum* could present this condition because of this protein responded the Cd heavy metal existence rather than Heat Shock Protein (HSP70) [25]. Protein metallothioneis (MT) could be used as biologic signer (biomarker) of Cd heavy metal in coastal areas [18]. In short, the research result in form of activation, concentration and expression of protein MT-1 on *Deadema setosum* is a biomonitoring model in molecular level that observing the biomolecular response of activation, concentration, and expression of protein MT-1 resulted by explanation of Cd heavy metal itself. Thus, data of biomonitoring result can be used as platform to take decision and be appropriate in environment management, and as risk assessment to the environment also [44].

V. CONCLUSION

The examination result of protein MT-1 by using imonohistochemistry, measurement on concentration protein MT-1 with ELISA reader, and examination on expression protein MT-1 with western blotting showed an increase with the higher Cd heavy metal. On the other side, sea urchin *D. setosum* can be used as a biomonitoring tool to Cd heavy metal pollution in the sea. It is emphasized by the analysis result on effect of Cd heavy metal treatment to protein MT-1 on *D. setosum* that prove the increase of concentration protein MT-1 in compliance to the higher concentration of Cd heavy metal. This condition indicated that protein MT-1 on *D.setosum* can be used as a biomonitoring heavy metal pollution in the sea on molecular level.

ACKNOWLEDGMENTS

In this opportunity, the writer would like to thank the Head of Laboratory UPT Conservation of Sea Biota Office, LIPI in Ambon, Indonesia with staffs and also the Head of Medical Physiology Laboratory at Brawijaya University, Indonesia, for supporting facilities to do research. Our great gratitude to Ditjen of Higher Education of National Education Ministry that has given BPPS for the writer in order to completing his Doctoral Program (S3) at Biology Education, Post Graduated Program at Official University State of Malang in the year of 2010/2011.

REFERENCES

[1] Nordic, *Cadmium Review*. Denmark: Prepared by COWI A/S on behalf of the Nordic Council of Ministers (2003).
[2] Gbaruko, B.C and O.U. Friday, Bioaccumulation of heavy metals in some fauna and flore, Email: bgbaruko@yahoo.com (2007).
[3] Zhou, Q., J.B. Zhang, J.J. Fu, J.B. Shi and G.B. Jiang, Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem, *Anal Chim Acta*, 606, 2008, 135–150.
[4] Schutzendubel, A., P. Schwanz, T. Teichmann, K. Gross, R. Langenfeld-Heyser, D.L. Godbold and A. Polle, Cadmium-Induced Changes in Antioxidative Systems, Hydrogen Peroxide Content, and Differentiation in Scots Pine Roots1, *Plant Physiology*, 127, 2001, 887–898.
[5] de Michele, R., E. Vurro, C. Rigo, A. Costa, L. Elviri, Di Valentine, M. Careri, M. Zottini, S. di Topi and F.L. Schiavo, Nitric oxide is involved in cadmium-induced Programme cell death in Arabidopsis suspension cultures, *Plant Physiology*, 150, 2009, 217-228.
[6] Flora, S.J.S., M. Mittal and A. Mehta, Heavy metal induced oxidative stres & its possible reversal by chelation therapy, *Indian J. Med*. Res, 128, 2008, 501-523.
[7] Agnello, M., S. Filosto, R. Scudiero, A.M. Rinaldi and M.C. Roccheri, Cadmium induces an apoptotic response in sea urchin embryos, *Cell Stress & Chaperones*, 12, 2007, 44–50.
[8] Agnello, M., S. Filosto, R. Scudiero, A.M. Rinaldi and M.C. Roccheri, Cadmium accumulation induces apoptosis in *P. Lividus* embryos, *Caryologia*, 59, 2006, 403-408.
[9] Patrick, L, Toxic metals and antioxidants. Part II the role of antioxidant in arsenic and cadmium toxicity – toxic metals part II. *Alternativer Medicine* Review, 8, 2003, 106-128.
[10] Krichah, R., K.B. Rhouma, D. Hallègue, O. Tébourbi, V. Joulin, D. Couton, and M. Sakly, Acute Cadmium Administration Induces Apoptosis in Rat Thymus and Testicle, but not Liver, *Polish Journal of Environmental Studies*, 12, 2003, 589-594.
[11] Rico, L.G., C.F. Felix, R.R. Burguenso and M.J. Marini, Determination of cadmium and zinc and its relathionship to metalloghionein level in swine kidney, *Rev. In. Contant Ambient*, 18, 2002, 157-162.
[12] Olive, L., J. Sisk and J. Bressler, Involvement of DMT1 in uptake of Cd in MDCK cells" role of protein kinase C. *Am. J. Physiol Cell.*, 281, 2001, 793-800.

[13] Ohta, H., Y. Yamauchi, M. Nakakita, H. Tanaka, S. Asami, Y. Seki and H. Yoshikawa, Relationship between Renal Dysfunction and Bone Metabolism Disorder in Male Rats after Long-Term Oral Quantitative Cadmium Administration, *Industrial Healthol*, 38, 2000, 339–355.

[14] Cohen, C.K., C.F. Tama, D.F. Garvin and L.V. Kochian, The role of iron-deficiency stres responses in stimulating Heavy metal transport in plants, *Plant Physiol*, 116, 1988, 1063-1072.

[15] Maanan, M, Biomonitoring of heavy metals using *Mytilus galloprovincialis* in Safi coastal waters, Morocco, *Environmental Toxicology*, 22, 2007, 525-531.

[16] Pal, M., E. Horvarth, T. Janda, E. Paldi and G. Szalai, Physiological changes and defense mechanisme induced by cadmium stress in maize. Review article, *J. Plant. Nutr. Soil Sci*, 159**,** 2006, 230-246.

[17] Soualili, D., P. Dubois, P. Gosselin, P. Pernet and M. Guillou, Assessment of Seawater Pollution by Heavy Metals in the Neighbourhood of Algiers: use of sea urchin, *Paracentrotus lividus*, as Bioindicator, *Journal of Marine Science*, 2, 2008, 1-25.

[18] Russo, R., R. Bonaventura, F. Zito, H. Schroder, I. Muller, W.E.G. Muller and V. Matranga, Stress to cadmium monitored by metallothionein gene induction in Paracentrotus lividus embryos, *Cell Stress & Chaperones*, 8, 2003, 232-231.

[19] Rumahlatu, D, Respon perilaku bulu babi *Deadema setosum* terhadap logam berat kadmium (Behavioral responses of sea urchins *Deadema setosum* the heavy metal cadmium), *Bumi Lestari Journal of Environment*, 12, 2012a, 45-54.

[20] Rumahlatu, D, Konsentrasi Logam Berat Kadmium pada Air, Sedimen, dan *Deadema setosum* di Perairan Pulau Ambon (Concentration of Heavy Metal Cadmium In Water, Sediments and *Deadema setosum* (Echinodermata, Echinoidea) in the waters of Ambon Island, *Indonesia Journal of Marine Science*, 16, 2011, 78-85.

[21] Danis, B., O. Cotret, J.L. Teyssié, P. Bustamante, S.W. Fowler and M. Warnau, Bioaccumulation of PCBs in the sea urchin *Paracentrotus lividus*: seawater and food exposures to a 14C-radiolabelled congener (PCB#153), *Environmental Pollution*, 135, 2005, 11-16.

[22] Flammang, P., M. Warnau, A. Temara, D.J.W. Lane and M. Jangoux, Heavy metals in *Deadema setosum* (Echinodermata Echinoidea) from Singapore coral reefs, *J. Sea Res*, 38, 1997, 35-45.

[23] Rumahlatu, D: Aktivitas Makan dan Pertumbuhan Bulu babi *Deadema setosum* Akibat Paparan Logam Berat Kadmium (Feeding Activity and Growth of Sea urchins *Deadema setosum* Due to Heavy Metal Cadmium Exposure). *Indonesia Journal of Marine Science*., 17, 2012b, 183-188.

[24] Rainbow, P.S., M. Wolowicz, W. Fialkowski, B.D. Smith and A. Sokolowski, Biomonitoring of trace metals in the Gulf of Gdansk, using mussels (*Mytilus trossulus*) and barnacles (*Balanus improvisus*), *Water Research*, 34, 2000, 1823-1829.

[25] Roccheri, M.C., M. Agnello, R. Bonaventura and V. Matranga, Cadmiun induces the expression of specific stress proteins in sea urchin embryos, *Biochemical and Biophysical Research Communications*, 321, 2004, 80–87.

[26] Choudhury, S and S.K Panda, Induction of oxidative stres and ultrastructural chages in moss *Taxithelium nepalense* (Schwaegr) Broth, unde lead and arsenic phytotoxicity, *Current Science*, 87, 2004, 342-348.

[27] Saydam, N., T.K. Adams, F. Steiner, W. Schaffner and J.H. Freedman, Regulation of Metallothionein transcription by the metal-responsive transcription factor MTF-1, *The Journal of Biochemical Chemistry*, 277, 2002, 20438-20448.

[28] Brulle, F., G. Mitta, R. Leroux, S. Lemiere, A. Lepretre and F. Vandenbulcke, The strong induction of metallothionein gene following cadmium exposure transiently affects th expression of many genes in Eisenia fetida: A Trade-off mechanism, *Compaparative Biochemistry and Physiology*, 144, 2007, 334-341.

[29] Thirumoorthy N, K.T.M. Kumar, A.S. Sundar, L. Panayappan and Chatterjee, Metallothionein: An overview. *World Journal of Gastroenterology*, 13, 2007, 993-996.

[30] Wu, J.P., B.Y. Ma, H.W. Ren, L.P. Zhang, Y. Xiang and M.A. Brown, Characterization of metallothioneins (MT-I and MT-II) in yak, *J Anim Sci*, 85, 2007, 1357-1362.

[31] Ogra, Y., S. Onishi, A. Kajiwara, A. Hara and K.T. Suzuki, Enhancement of Nuclear Localization of Metallothionein by Nitric Oxide, *Journal of Health Science*, 54, 2008, 339-342.

[32] Carpene, E., G. Andreani and G. Isani, Metallothionein functions and structural characteristcs. *Journal of Trace Elements in Medicine and Biology*, 21,2007, 35-39.

[33] Pearce, L.L,. R.E. Gandley, W. Han, K. Wasserloos, M. Stitt, A.J. Kanai, M.K. McLaughlin, B.R. Pitt and E.S. Levitan, Role of metallothionein in nitric oxide signaling as revealed by a green fluorescent fusion protein, *PNAS*, 97, 2008, 477–482.

[34] Wu, J.P and H.C. Chen, Metallothionein induction and heavy metal accumulation in white shrimp Litopenaeus vannamei exposed to cadmium and zinc, *Comparative Biochemistry and Physiology*, 140, 2005, 383–394.

[35] Lequin, R.M, Enzyme Immunoassay (EIA)/Enzyme-Linked Immunosorbent Assay (ELISA), *Clinical Chemistry*, 51, 2005, 2415–2418.

[36] Young, J and M. Hongbao, Practical Technique of Western Blotting. *The Journal of American Science*, 4, 2008, 1-4.

[37] Nielsen, A.E., A. Bohr and M. Penkowa, The balance between life and death of cell: role of metallothioneins, *Biomarker Insights*., 1, 2006, 99-111.

[38] Kimura, T and N. Itoh: Function of metallothionein in gen expression and singnal transduction, Newly found protective role of metallothionein, *Journal of Health Science*, 54, 2008, 251-260.

[39] Formigari, A., P. Alberton, V. Cantale, V. De Nadal, M. Feltrin, S. Ferronato, A. Santon, L. Schiaovon and P. Irato, Relationship between metal transcription fakctor-1 and Zinc in resistance to metals producing free radicals, *Current Chemical Biology*, 2, 2008, 256-266.

[40] Langmade, S.J., R. Ravindra, P.J. Daniels and Andrews, The regulation factor MTF-1 mediates metal regulation of the mouse ZnT1 gene, *The Journal of Biological Chemistry*, 275, 2000, 34803-34809.

[41] Martin, L.M., J.E. Hesketh, J.H. Beattie and H.M. Wallacet, Influence of metallothionein-1 localization on its function, *Biochem J*., 355, 2001, 463-479.

[42] Florianczyk, B, Metallothioneins and its role in metal regulation, binding reactive oxygen species, apoptosis and cell differentiation, *Journal of Pre-clinical and clinical research*, 1, 2007, 106-108.

[43] Werner, I., S.L. Clark and D.E. Hinton, Biomarkers aid understanding of aquatic organism responses to environmental stressors, *California Agriculture*, 57, 2003, 110-115.

[44] Angerer J., M.G.Bird, T.A. Burke, N.G. Doerrer, L. Needham, S.H. Robinson, L. Sheldon and H. Zenick, Strategic biomonitoring initiatives: moving the science forward, *Toxicological Science*, 93, 2006, 3-10.

[45] Kamrin, M.A: Biomonitoring Basics. A resport from biomonitoringinfo.org. *Environmental Health Research Foundation*. Institute for Environmental Toxicology, Michigan State University, (2004).