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Research Article
Effect of Mercury ($HgCl_2$) Sub-chronic Doses Exposure in *Tubifex* sp.

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Abstract

Background and Objective: Mercury is the most toxic heavy metal found in the environment. All aquatic organism has the potential to accumulate heavy metal on their body including *Tubifex* sp. Metallothionein has an important role in heavy metal detoxification mechanism which synthesized in each organism body. The aim of this study was to observe the mortality percentage, mercury ($HgCl_2$) levels and mercury metallothionein (Hg-MT) levels in *Tubifex* sp. after 7 days of exposure at sub-chronic doses. **Materials and Methods:** The experimental method was used in this study. The mercury different sub-chronic doses (0, 12.5, 25, 37.5, 50 and 100%) were treated to *Tubifex* sp. triplicate for accumulative 7 consecutive days. **Results:** The result showed that the highest mortality (%) of *Tubifex* sp. was 100% sub-chronic dosage of mercury exposure with value 98.7±1.2% and the lowest mortality (%) was 0% sub-chronic dosage of mercury exposure with value 16.7±1.5%. The highest level of mercury and Hg-MT were in the 100% sub-chronic dosage of mercury exposure with value 4930±88.9 and 0.117±0.011 μg L^{-1}, respectively and the lowest level were 0% sub-chronic dosage of mercury exposure with value 13.3±5.8 and 0.019±0.009 μg L^{-1}, respectively. The Hg-MT level was not significantly different between 100 and 50% of sub-chronic doses, then the mortality (%) and $HgCl_2$ level in *Tubifex* sp. body were significantly different in 100% compared to all treatment. **Conclusion:** So, it was concluded that the maximum capability of *Tubifex* sp. in synthesizing Hg-MT was 50% sub-chronic dosage of mercury exposure statistically with the highest mortality (%) and mercury level in *Tubifex* sp. was 100% sub-chronic dosage of mercury exposure.

Key words: Aquatic pollution, mercury exposure, metallothionein, sub-chronic dosage, sub-chronic, *Tubifex* sp.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mercury is one of the non-essential metal which able to induce MT synthesis[1,2]. Commonly, mercury is divided into several forms; inorganic mercury, elemental or metallic (Hg^0) mercury, mercurous (Hg_2^{++}) mercury, mercuric (Hg^{++}) mercury, and organic mercury[3]. Mercury considered as the most toxic heavy metal in the environment which is caused by anthropogenic activities including mining, agriculture activities, municipal wastewater discharge and industrial wastewater discharge[4,5]. When toxic metal spread to the natural ecosystem, metal ions will accumulate in the body through direct or indirect uptake such as through food chains. Heavy metal metabolism inside the organism body is the main factor to determines metal toxicity[6].

Metallothionein (MT) is a ubiquitous low molecular weight protein which capable of binding metal which behaviour is dominated by the chemistry of the thiol (-SH) group[7,8]. The MT exist in bacteria, plants, invertebrates and vertebrates[9-11] and also play an important role to cell protected faced high levels of essential metals, as well as in detoxifying non-essential metals such as; mercury and cadmium[12,13]. The ability is related to heavy metal detoxification process inside the organism that secreted metallothionein[14].

All aquatic invertebrates will absorb and accumulate metals in their body whether is needed or not for metabolism[15]. *Tubifex* sp. is kind of freshwater Oligochaeta group *Tubificid* worms, which lives in the water or sediment interface. *Tubifex* sp. which lives in the contaminated environment can be a bio-indicator of a toxic pollutant for higher organisms in a trophic chain[16]. Beside that *Tubificid* worms is an essential part of the detritus food chain which potential as bio-accumulator of heavy metal to the higher animal or organism and *Tubificid* worms used as a test organism for bioassay sediments[2].

In present, several laboratory and field studies focused on MT response under mercury exposure on fish[17-20]. Besides fish, some studies used *Tubifex tubifex* on heavy metal observation[16,21,22], but there are no studies about the exposure of mercury sub-chronic doses by *Tubifex* sp. Based on this reason, the information about sub-chronic doses of mercury exposure was needed. The aim of this study was to observe the mortality (%), mercury levels and Hg-MT levels in *Tubifex* sp., whole body after 7 days of exposure at varying mercury sub-chronic doses.

MATERIALS AND METHODS

Preliminary research: A method used in the research was an experimental method. This study was the continuation of previous research which involves toxicity test which obtained LC_{50} 48 h of $HgCl_2$ on *Tubifex* sp. at dose 83.15±3.89 μg L^{-1}. The test animals in this study were *Tubifex* sp. worm. The treatment in this study was different doses of $HgCl_2$ (0, 12.5, 25, 37.5, 50 and 100%). The dosage determination based on the LC_{50} 48 h that has done before and each dosage treatment repeated three times.

Research preparation: The sample was *Tubifex* sp. from water contaminated with industrial waste in Pasuruan Regency, East Java, Indonesia. The samples were taken directly than separated from the sediment and rinsed with flowing water. The samples were stored in the flowing water and were carried out to the Hydrobiology Laboratory, Faculty of Fisheries and Marine Science, University of Brawijaya, Malang, Indonesia for further assay. Before assay, these samples were acclimatized in the laboratory for 2 weeks without feeding. This study was held from August, 2017-August, 2018 then the sample was exposure of varying mercury sub-chronic doses in 7 consecutive days.

Sub-chronic of mercury level assay: The initial step of the sub-chronic assay was prepared the *Tubifex* sp. from preparation sample which acclimatized before, then the samples were moved to the 1 L tank with 200 mL water each (50 worms per tank) and were second acclimatized around 48 h and used the healthy worms. Sub-chronic doses were prepared with dose 0, 12.5, 25, 37.5, 50 and 100% which followed LC_{50} 48 h for 100% as 83.15±3.89 μg L^{-1}. Each dose was performed in triplicate. The samples were treated followed different doses and observed everyday for 7 days, respectively, with changed the 75% of water while maintaining the initial dose every day. The test organisms in this study followed by Katzung[23], which standard 10% of *Tubifex* sp., worms-life expectancy. The mortality of *Tubifex* sp., was carried out in every day and made sure the worm was considered to be dead with no response after 10 sec with a bit of nuisance. The dead worms were taken away from the tank by using a dropper. *Tubifex* sp., which still alive did continue observation until the final day was counted the mortality rate then collected to measure the mercury level then Hg-MT level on the *Tubifex* sp., whole body. Determination of mercury level was carried out by using Atomic Absorption Spectrophotometry (AAS) method and Hg-MT level was determined by Enzyme-Linked Immunosorbent Assay (ELISA) method using ELISA Kit Fish MT-2.

Statistical analysis: Data were analyzed by one-way analysis of variance (ANOVA) in SPSS ver. 16.0 software with a confidence level of 95% ($\alpha<0.05$) which followed the Duncan test. Data were expressed as a mean±standard deviation (SD).

RESULTS

Mortality rate of *Tubifex* sp.: The mortality (%) of *Tubifex* sp., after 7 days exposed to $HgCl_2$ ranged from 16.7±1.5 to 98.7±1.2%. Figure 1 showed the mortality (%) of *Tubifex* sp., worms after 7 days of $HgCl_2$ exposure. The highest of mortality at 100% of sub-chronic mercury dose and the lowest in non-exposed worms (0% of sub-chronic mercury dose). The mortality of *Tubifex* sp., after 7 days exposed to $HgCl_2$ showed an increase in higher doses. Based on the ANOVA result that the mortality (%) of *Tubifex* sp., significantly different at 100% sub-chronic dose of mercury compared to all treatment.

Mercury level in *Tubifex* sp.: The mercury levels in *Tubifex* sp., whole body after $HgCl_2$ exposure for 7 days ranged from 13.3±5.8 to 4930±88.9 μg L^{-1}. Highest mercury level at a dose of $HgCl_2$ 100% and the lowest mercury level at a dose of $HgCl_2$ 0% (Fig. 2). Based on the ANOVA results, the treatment of $HgCl_2$ concentration affects the mercury levels in the body's *Tubifex* sp., with a significant difference at 100% sub-chronic dose of mercury compared to all treatment. Figure 2 showed the mercury level in the bodies of *Tubifex* sp., worm increasing at higher doses.

Hg-MT levels in *Tubifex* sp.: Mercury Metallothionein (Hg-MT) levels in *Tubifex* sp., which exposed with $HgCl_2$ in sub-chronic level with variety of mercury concentrations (0, 12.5, 25, 37.5, 50 and 100% from LC_{50} dose) showed that 100% $HgCl_2$ exposure yields highest MT level (0.117±0.011 μg L^{-1}) compared to other dosage treatments. *Tubifex* sp., which not exposed to $HgCl_2$ (0% $HgCl_2$ exposure) had an MT level of 0.019±0.009 μg L^{-1} which was the lowest level among other treatments. ANOVA results showed that Hg-MT in *Tubifex* sp., body was not significantly different between 50 and 100% of mercury sub-chronic dose, but had significantly different from the others treatment (Fig. 3).

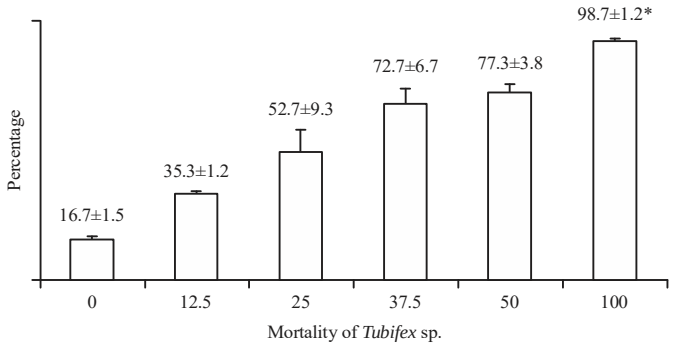

Fig. 1: Mortality (%) of *Tubifex* sp., after sub-chronic $HgCl_2$ exposure for 7 days
All treatment data were compared to each treatment and standard of deviation (SD) as the error bars with $\alpha<0.05$ (*Significant)

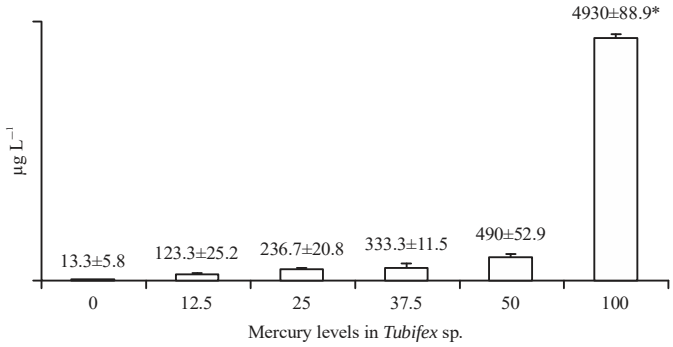

Fig. 2: Mercury levels in *Tubifex* sp., after subchronic $HgCl_2$ exposure for 7 days
All treatment data were compared to each treatment and standard of deviation (SD) as the error bars with $\alpha<0.05$ (*Significant)

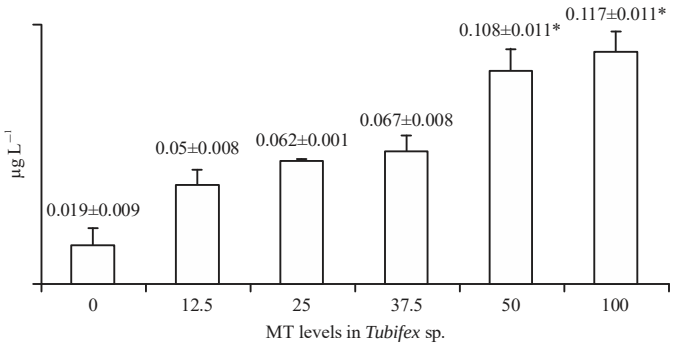

Fig. 3: Metallothionein levels in *Tubifex* sp., after sub-chronic $HgCl_2$ exposure for 7 days
All treatment data were compared to each treatment and standard of deviation (SD) as the error bars with $\alpha<0.05$ (*Significant)

DISCUSSION

It was observed that the mortality (%) increases along with the high dose of mercury. It can also be proved by the high mercury level in *Tubifex* sp., body in the higher dose of mercury. *Tubifex* sp. has the ability in tolerating to mercury which absorbed in its body. At the highest dose, *Tubifex* sp., could not tolerate the mercury over than 48 h. It is suspected that 48 h was a time limit for *Tubifex* sp., which can tolerate the mercury exposure. A previous study explained that the distribution of organic mercury after ingestion to the blood compartment is complete in 30 h[24]. The quantification of heavy metals in the organism body can exceed from the limits of individual adaptive abilities[25,26]. Each individual has different tolerate of heavy metals, especially for mercury. Based on Environmental Quality Standards, it was reported that *Ophryotrocha diadema* tolerates in 28 days, *Ctenodrilus serratus* tolerates in 21-31 days with sub-chronic exposure of mercury source saltwater. While at lower doses the *Tubifex* sp., mortality showed gradually along with the lower dose of mercury. It happened because, at lower doses, *Tubifex* sp., could tolerate and adapt to mercury exposure[27]. It was reported that metallic mercury is a fat-soluble which easy to across the alveolar cell barrier and oxidize to inorganic mercury and combines with protein and showed a cumulative effect. Mercury exists in several forms: Metallic, inorganic and organic compound. Metallic mercury absorbed through the respiratory tract and hardly absorbed in gastrointestinal (GI) tract, inorganic mercury commonly absorbed through the respiratory tract, in small value is also absorbed through skin (3-4%) or GI tract (2-10%) and methylmercury is a major type of organic mercury with the most toxic properties on the human body, commonly, its found in methylmercury and dimethylmercury which easy to absorb into GI tract ($\geq$95%) and respiratory tract (80%)[28]. Based on one-way ANOVA statistically mortality (%) of *Tubifex* sp. and Mercury level in *Tubifex* sp., after 7 days exposure with various sub-chronic doses of mercury showed that except 100%, other doses showed not significantly different compared to all treatment, which means that at the average mortality of *Tubifex* sp. and the amount of mercury accumulation in its body were the same. These results differed from the level of Hg-MT in *Tubifex* sp., body which exposed with various of sub-chronic doses of mercury. Metallothionein (MT) is a low molecular weight protein with a high cysteine content strong affinity for heavy metals[29]. The MTs in aquatic organisms is focusing on their function as like biomarker for biomonitoring programs of heavy metals and heavy metal detoxification to protect the tissues from various forms of oxidative injuries and transferring of essential metals[14,30,31]. Different metal accumulation and MT levels showed various types of fish and depend on the organ/tissue as a function of its biochemical and physiological features[32]. In the other hand, MT induction also depends on the exposure duration and on the metal concentration[33]. The findings of current study were supported by Carricavur *et al.*[34], in the polychaetes which exposed by Cd showed MT levels increased significantly only for 1300 and 2000 μg Cd L^{-1} in comparison to controls. It is necessary to confirm the mercury (Hg) transformation in the *Tubifex* sp., body in the further research.

CONCLUSION

Mercury accumulated in *Tubifex* sp. was directly proportional with $HgCl_2$ level and Hg-MT synthesis in their body. The mortality rate, mercury level and Hg-MT level of *Tubifex* sp., worms increased at higher sub-chronic mercury dose. Level of mercury metallothionein (Hg-MT) was maximum synthesis at 50% of sub-chronic $HgCl_2$ exposure.

SIGNIFICANCE STATEMENT

This study discovers the mortality (%), mercury ($HgCl_2$) levels and mercury metallothionein (Hg-MT) levels in *Tubifex* sp. after 7 days of exposure at sub-chronic doses. This study will help the researcher to uncover the critical area related to the toxicity of mercury doses and it was find that the Mercury accumulated in *Tubifex* sp., was directly proportional with $HgCl_2$ level and Hg-MT synthesis in their body that many researchers were not able to explore.

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REFERENCES

1. Romeo, M., P. Hoarau, G. Garello, M. Gnassia-Barelli and J.P. Girard, 2003. Mussel transplantation and biomarkers as useful tools for assessing water quality in the NW Mediterranean. Environ. Pollut., 122: 369-378.
2. Amiard, J.C., C. Amiard-Triquet, S. Barka, J. Pellerin and P.S. Rainbow, 2006. Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers. Aquat. Toxicol., 76: 160-202.
3. Bernhoft, R.A., 2012. Mercury toxicity and treatment: A review of the literature. J. Environ. Public Health, Vol. 2012. 10.1155/2012/460508.
4. Chen, C.W., C.F. Chen and C.D. Dong, 2012. Distribution and accumulation of mercury in sediments of Kaohsiung River Mouth, Taiwan. APCBEE Procedia, 1: 153-158.
5. Jan, A.T., I. Murtaza, A. Ali and Q.M.R. Haq, 2009. Mercury pollution: An emerging problem and potential bacterial remediation strategies. World J. Microbiol. Biotechnol., 25: 1529-1537.
6. Yoo, H., H. Lee and W. Jeong, 2002. Preparation of ion exchanger from waste paper cup and removal characteristics of heavy metal [J]. J. Kor. Environ. Sci., 11: 993-999.
7. Romero-Isart, N. and M. Vasak, 2002. Advances in the structure and chemistry of metallothioneins. J. Inorg. Biochem., 88: 388-396.
8. Mieiro, C.L., L. Bervoets, S. Joosen, R. Blust, A.C. Duarte, M.E. Pereira and M. Pacheco, 2011. Metallothioneins failed to reflect mercury external levels of exposure and bioaccumulation in marine fish-considerations on tissue and species specific responses. Chemosphere, 85: 114-121.
9. Vasak, M., 2005. Advances in metallothionein structure and functions. J. Trace Elem. Med. Biol., 19: 13-17.
10. Henkel, G. and B. Krebs, 2004. Metallothioneins: zinc, cadmium, mercury and copper thiolates and selenolates mimicking protein active site features-structural aspects and biological implications. Chem. Rev., 104: 801-824.
11. Coyle, P., J.C. Philcox, L.C. Carey and A.M. Rofe, 2002. Metallothionein: The multipurpose protein. Cell Mol. Life Sci., 59: 627-647.
12. Roesijadi, G., 1996. Metallothionein and its role in toxic metal regulation. Comp. Biochem. Physiol. Part C: Pharmacol., Toxicol. Endocrinol., 113: 117-123.
13. Viarengo, A., B. Burlando, N. Ceratto and I. Panfoli, 2000. Antioxidant role of metallothioneins: a comparative overview. Cell. Mol. Biol., 46: 407-417.
14. Hall, J.L., 2002. Cellular mechanisms for heavy metal detoxification and tolerance. J. Exp. Bot., 53: 1-11.
15. Rainbow, P.S., 2002. Trace metal concentrations in aquatic invertebrates: Why and so what? Environ. Pollut., 120: 497-507.
16. Luszczek-Trojnar, E., K. Sroka, A. Klaczak, M. Nowak and W. Popek, 2014. Bioaccumulation and purification of cadmium in *Tubifex tubifex*. Turk. J. Fish. Aquat. Sci., 14: 939-946.
17. Gonzalez, P., Y. Dominique, J.C. Massabuau, A. Boudou and J.P. Bourdineaud, 2005. Comparative effects of dietary methylmercury on gene expression in liver, skeletal muscle and brain of the zebrafish (*Danio rerio*). Environ. Sci. Technol., 39: 3972-3980.
18. Bebianno, M., C. Santos, J. Canario, N. Gouveia, D. Sena-Carvalho and C. Vale, 2007. Hg and metallothionein-like proteins in the black scabbardfish *Aphanopus carbo*. Food Chem. Toxicol., 45: 1443-1452.
19. Jebali, J., M. Banni, H. Gerbej, H. Boussetta, J. Lopez-Barea and J. Alhama, 2008. Metallothionein induction by Cu, Cd and Hg in *Dicentrarchus labrax* liver: Assessment by RP-HPLC with fluorescence detection and spectrophotometry. Mar. Environ. Res., 65: 358-363.
20. Oliveira, M., I., Ahmad, V.L. Maria, A. Serafim, M.J. Bebianno, M. Pacheco and M.A. Santos, 2010. Hepatic metallothionein concentrations in the golden grey mullet (*Liza aurata*)-relationship with environmental metal concentrations in a metal-contaminated coastal system in Portugal. Mar. Environ. Res., 69: 227-233.
21. Rathore, R.S. and B.S. Khangarot, 2002. Effects of temperature on the sensitivity of sludge worm *Tubifex tubifex* (Muller) to selected heavy metals. Ecotox. Environ. Saf., 53: 27-36.
22. Kaonga, C.C., J. Kumwenda and H.T. Mapoma, 2010. Accumulation of lead, cadmium, manganese, copper and zinc by sludge worms; *Tubifex tubifex* in sewage sludge. Int. J. Environ. Sci. Technol., 7: 119-126.
23. Katzung, B.G. and D.C. Furst, 2007. Non Steroidal Anti-Inflammatory Drugs. In: Basic and Clinical Pharmacology, Katzung, B.G. (Ed.). 10th Edn., McGraw-Hill Medical, USA., ISBN-13: 9780071451536, pp: 591.

24. Gupta, R.C., 2012. Veterinary Toxicology: Basic and Clinical Principles. Academic Press, USA., ISBN: 9780123859266, Pages: 1454.
25. Yap, C.K., A. Ismail and S.G. Tan, 2009. Effect of body size on heavy metal contents and concentrations in green-lipped mussel *Perna viridis* (Linnaeus) from Malaysian coastal waters. Pertanika J. Sci. Technol., 17: 61-68.
26. Li, X., L. Liu, Y. Wang, G. Luo and X. Chen *et al*., 2012. Integrated assessment of heavy metal contamination in sediments from a coastal industrial basin, NE China. PloS One, Vol. 7. 10.1371/journal.pone.0039690.
27. EQS., 2006. Common implementation strategy for the water framework directive. Priority Substance No. 21, Mercury and its Compounds, CAS-No. 7439-97-6.
28. Ye, B.J., B.G. Kim, M.J. Jeon, S.Y. Kim and H.C. Kim, 2016. Evaluation of mercury exposure level, clinical diagnosis and treatment for mercury intoxication. Ann. Occup. Environ. Med., Vol. 28. 10.1186/s40557-015-0086-8.
29. Shamsi, T.N. and S. Fatima, 2014. Metallothionein: Classification, biochemical features and clinical applications. J. Proteins Proteomics, 5: 25-33.
30. Baird, S.K., T. Kurz and U.T. Brunk, 2006. Metallothionein protects against oxidative stress-induced lysosomal destabilization. Biochem. J., 394: 275-283.
31. Wang, W.C., H. Mao, D.D. Ma and W.X. Yang, 2014. Characteristics, functions and applications of metallothionein in aquatic vertebrates. Front. Mar. Sci., Vol. 34. 10.3389/fmars.2014.00034
32. De Boeck, G., T.T. Ngo, K. van Campenhout and R. Blust, 2003. Differential metallothionein induction patterns in three freshwater fish during sublethal copper exposure. Aquat. Toxicol., 65: 413-424.
33. Hamza-Chaffai, A., R. Cosson, C. Amiard-Triquet and A. El Abed, 1995. Physico-chemical forms of storage of metals (Cd, Cu and Zn) and metallothionein-like proteins in gills and liver of marine fish from the Tunisian coast: Ecotoxicological consequences. Comp. Biochem. Physiol. Part C: Pharmacol., Toxicol. Endocrinol., 111: 329-341.
34. Carricavur, A.D., L.C., Boudet, M.B. Romero, P. Polizzi, J.E. Marcovecchio and M. Gerpe, 2018. Toxicological responses of *Laeonereis acuta* (Polychaeta, Nereididae) after acute, subchronic and chronic exposure to cadmium. Ecotoxicol. Environ. Saf., 149: 217-224.