INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 12, No. 5, p. 189-195, 2018
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Toxicity of lead (Pb) at pH 6.5 and 8.5 to the whiteleg shrimps *Litopenaeus vannamei*

Muhammad Arif Asadi*, Defri Yona, Riizky Ade Pratama

Department of Marine Science, University of Brawijaya, Malang, Indonesia

Article published on May 30, 2018

Key words: Pb, Toxicity, pH, *L. vannamei*, LC_{50}.

Abstract

Lead accumulation in the marine environment is the result of anthropogenic use. The precipitation characteristic of lead elevates the risk of this metal exposure to the benthic communities. The study aimed to access the influence of acidic and basic environment on the toxicity of lead to the benthic organism, the whiteleg shrimp *L. vannamei* through acute toxicity test. The median lethal concentration was evaluated using probit analysis, and the univariate analysis of variance was used to determine which factors more likely influenced the mortalities of the test animal. At both pH 6.5 and 8.5, the higher Pb concentration resulted higher mortality of the test animals, and at 1000 ppm, all the shrimps were dead even at earliest observation. The LC_{50} values at pH 6.5 and 8.5 were 216.57 ppm and 259.84 ppm respectively in which Pb metal pollution at both pH is still practically non-toxic to the whiteleg shrimps, *L. vannamei*. Furthermore, based on the univariate ANOVA, both pH as well as the interaction of Pb and both pH do not significantly cause the mortality of the test animal as *F*-stat < *F*-table. The only factor that significantly caused the mortality of the shrimps was Pb concentration as *F*-stat > *F*-table. Therefore, there is no synergistic toxicity effects of Pb in both pH 6.5 and 8.5 to the whiteleg shrimps, *L. vannamei*.

***Corresponding Author:** Muhammad Arif Asadi ✉ asadi@ub.ac.id

Introduction

The production, use, and disposal of lead (Pb) have significantly polluted the earth's soil, body of waters, and marine environment. The input of the metal to marine environments is mainly through release into the atmosphere, and anthropogenic Pb is the main source of the pollution in the ocean's water column and sediment which it accumulates over time (Muñoz *et al*., 2004; Szmytkiewicz and Zalewska, 2014). The global anthropogenic emission of Pb is higher than that of natural emissions by a factor of 28 (332×10^3 ton y^{-1} and 12×10^3 ton y^{-1} respectively), which the combustion of fossil fuels is the major input to the environment (Harrison, 2001).

In marine organisms, some populations are more sensitive than others, and benthic communities are prone to Pb pollution as the precipitation characteristic of the metal increase its bio-availability toward the organisms (Valdes and Calderon, 2012). Sea snail (*Rapana venosa*), for example, accumulate Pb at much higher levels than other animals found in the water column. The animals mainly feed on other benthic organisms like oysters, mussels, and other bivalves, which these filter feeders have already accumulated the metal by straining food particles from water, thus the level of Pb in the tissues of the snails increase at successively higher levels through biological magnification (Baltas *et al*., 2017).

The whiteleg shrimp, *L. vannamei* is also benthic invertebrate living in tropical marine habitats and feeding on benthic detritus, bivalves, worms, and other crustaceans (Dall *et al*., 1991). Tissues samples of this crustacean indicate evidence of some metals bioaccumulation, increasing environmental concern regarding these inorganic contaminants (Silva *et al*., 2016). In other crustacean species, the accumulation of Pb occurs in species-specific manner; for example, isopod crustacean species, *Armadillidium vulgare* and *Porcellio laevis* exhibit significant differences in the metal accumulation as well as in their lethality which the former species accumulates higher level of Pb and has higher level of lethal concentration (Mazzei *et al*., 2014).

L. vannamei is suitable organism for ecological testing not only because it is an important species for inshore fishermen and aquaculture in many countries but also it is feeding behavior as benthic organisms which increases its vulnerability toward Pb contaminants in the sediment (Mazzei *et al*., 2014; Rand, 1995; Szmytkiewicz and Zalewska, 2014). As calcifying organism, this species is also more vulnerable to climate change effects as it forms its carapace or exoskeleton out of chitin. The increasing emissions of anthropogenic carbon dioxide could decrease ocean pH and carbonate ion concentrations, leading the increase of shell's dissolution and elevating the difficulties of maintaining its calcium carbonate skeletons (Orr *et al*., 2005). Therefore, in this study, we would like to examine the effect of pH on the toxicity of Pb to the whiteleg shrimps, *L. vannamei*.

Materials and methods

L. vannamei maintenance.

The shrimps were obtained from a commercial shrimp hatchery in Paciran, Indonesia and were acclimated in the marine science laboratory, University of Brawijaya. The larvae were bought at the age of 5-6 days old and maintained until the age of 13 days old in a 150cm x 45cm aerated tank with natural seawater taken from Kondang Merak waters, East Java. The tank was adjusted to pH 7.5±0.2, salinity 21±0.5%, and temperature 25±0.5°C. The shrimps were fed with both brine shrimp and formulated feed which contain approximately 30 percent fishmeal and minimum 35 percent protein (Subramani and Michael, 2017). In order to reduce ammonia content, approximately a third of water in the tank was replaced daily with fresh natural seawater.

Test chemicals

In this experiment, the pH was adjusted by adding NaOH and HCL solution to increase and decrease the level of pH respectively. Meanwhile, lead used in this experiment was in the form of lead acetate trihydrate, $Pb\ (CH_3COO)_2.\ 3H_2O$, a colourless monoclinic crystalline substance which has atomic mass of 379.33 g mol^{-1} (Sun *et al*., 2014).

Using molar mass calculation, the Pb percentage in the lead acetate trihydrate compound is known to be 54.62%. Therefore, in order to prepare the Pb solution with concentration of 1000 ppm, 1.83g of lead acetate trihydrate compound was weighed and put into 1-L Erlenmeyer flask, natural seawater with pH 6.5 was then poured into the flask until reached water volume of 1000ml. The 1000 ppm of Pb solution with the pH 8.5 was also prepared with the same manner.

Preliminary testing

Prior to acute toxicity tests, preliminary testing was conducted to assure adequate survival control (≥80%) and to determine the exposure range concentration. In brief, ten triplicate of *L. vannamei* (10 days old) were exposed for 48 hours to Pb concentrations of 0, 0,1, 1, 10, 100, and 1000 ppm in 1-L Erlenmeyer flasks with 500 mL of aerated seawater. The shrimps were exposed at both pH 6.5 and 8.5, salinity 21±0.5%, temperature 25±0.5°C, and under dark/light regime of 8/16 hours with a ±2500 lux lamp. During this testing, the water qualities (pH, salinity, and temperature) were checked at every 6 hours, and the mortalities were counted. At the end of preliminary testing, the minimum and maximum threshold or the exposure range of Pb were determined which were between 10 and 1000 ppm. These values were further used to determine the Pb concentration for the acute toxicity experiments.

Acute toxicity testing

The experiments were performed in triplicate using standard static experiment described by Lee *et al.*, (2013), in which a total of 54 Erlenmeyer flasks were set up for the acute toxicity test. The Pb concentrations for this acute toxicity test were 0% (control), 17.7, 31.6, 56.2, 100, 177, 316, 562, and 1000 pm. Those concentrations were chosen as the results of the logarithmic computation of the Pb thresholds which had been determined earlier during the end of the preliminary testing. Meanwhile, the pH values for the acute toxicity test were using the same pH levels used in the preliminary testing. Those pH levels should not affect the mortality of the test animals that could interfere the results of the acute toxicity experiments in which the survival rate of *L. vannamei* is known under the pH levels between 5 and 9 (Furtado *et al.*, 2015). The other environmental parameters (salinity, temperature, and light/dark regime) were also set up at the same levels with the preliminary testing. At every 12 hours, the water qualities were measured and the mortality of the shrimps were counted. These mortality data were used to determine the lethal concentration 50 (LC_{50}) of Pb to the whiteleg shrimps, *L. vannamei* using Finney's probit analysis.

Data analysis

The experiments and data analysis were conducted in July-September 2017. The mortality in each treatment was calculated using Microsoft Excel 2010. LC_{50} calculation using Finney's probit analysis as well as the Univariate Approach: Analysis of Variance (ANOVA) were also computed using the same software. The univariate ANOVA was performed to determine whether pH levels or the Pb concentrations that were leading factors in the mortalities of the test animals.

Result and discussion

Acute toxicity test

The effects of Pb on the mortalities of *L. vannamei* varied depending on the Pb concentrations, pH values, and time exposures. At both pH 6.5 and 8.5, the mortalities of control were significantly lower than those of all the Pb exposure concentrations (ANOVA, Dunnet post hoc test, $p < 0.01$). At the concentration of 1000 ppm, all the test animals were died even at the first 12 hours exposure. Overall, Pb was more toxic at the pH 6.5 than that of 8.5. It was prominent at the higher concentration (316 and 562 ppm). Although, at the lower concentration, the mortalities seemed higher at the pH 8.5 than those of pH 6.5 (Table 1 and Table 2).

In order to predict the LC_{50} values of *L. vannamei* exposed to Pb at both pH 6.5 and 8.5, Probit linear regression models had been performed for 12-h to 96-h exposure. It was shown that both pH values had very high correlation for 96-h exposure (R^2=0.958 and 0.935 for pH 6.5 and 8.5 respectively). The Probit equations for 96-h exposure were $y = 1.9071x + 0.5468$ and $y = 1.8358x + 0.5694$ for pH 6.5 and 8.5 respectively (Fig. 1). Based on these equations, the 96-h LC_{50} values of pH 6.5 was significantly lower than that of pH 8.5 which were 216.57 ppm and 259.84 respectively.

Table 1. The average number of mortality of *L. vannamei* exposed to Pb concentration between 0 and 1000 ppm at pH 6.5.

Pb Concentration (ppm)	The average mortality				
	12 h	24 h	48 h	72 h	96 h
0	0	0.6	1.3	1.3	2
17.7	0.3	1	2	2.3	2.6
31.6	0.3	1.3	2	3	3
56.2	0.6	1.6	2	3	4
100	0.6	1.6	2.6	3.6	4.6
177	2.3	2.6	4.3	4.6	5.3
316	3.3	4.6	6	7.3	8.3
562	8.6	10	10	10	10
1000	10	10	10	10	10

Table 2. The average number of mortality of *L. vannamei* exposed to Pb concentration between 0 and 1000 ppm at pH 8.5.

Pb Concentration (ppm)	The average mortality				
	12 h	24 h	48 h	72 h	96 h
0	0	1	1	1.6	2
17.7	0.3	1.3	1.6	2.6	3
31.6	0.6	1.3	2	3	3
56.2	1.3	2	2.3	3	3.3
100	1.6	2.3	2.6	3.3	3.6
177	2.3	3.3	3.3	4.6	4.6
316	3.6	4	4.3	5.3	6.6
562	7.6	9	10	10	10
1000	10	10	10	10	10

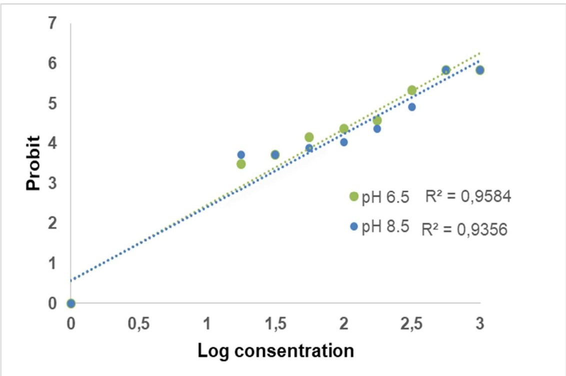

Fig. 1. The 96-h mortality probit curves of *L. vannamei* as function of log concentration of Pb at both pH 6.5 and 8.5.

The univariate analysis of variance

The univariate analysis of variance showed that pH did not have significant impact to the mortality of the test animals in which F-stat. (3.45) was significantly lower than F-table (4.11). Meanwhile, the result of the Pb concentration was F-stat. (87.13) > F-table (2.21). Therefore, Pb concentrations were the only factor that significantly impact the mortality of the whiteleg shrimps *L. vannamei* since the interaction between Pb and pH also did not elevate the mortality of the test animal (Table 3).

Table 3. Univariate statistics analysis of the mortality to determine which factors influenced the mortality of the test animals.

Source of Variation	df	*F*-stat.	*F*-table	*P* value
Pb concentrations	8	87.13	2.21	0.000
pH	1	3.45	4.11	0.093
Interaction	8	0.95	2.21	
Random	36			
Total	53			

Discussion

The discharge of lead could have devastating impact to marine environment as it accumulates over time. The adverse impact of this pollution is more prevalent in the bottom sediment of marine environment, in which its precipitation characteristic elevates the bio-availability of this metal toward benthic organisms (Szmytkiewicz and Zalewska, 2014; Valdes and Calderon, 2012). It is known that many filter feeders species and other marine benthic organisms accumulate this toxic metal at higher level than other marine organisms in the water column habitat (Baltas *et al.*, 2017). As a benthic organism, the whiteleg shrimps, *L. vannamei* could also suffer from this metal pollution.

This study aimed to assess the impact of Pb as well as its interaction with acidic and basic environment to *L. vannamei* by using acute toxicity test. In basic or acidic environment, toxicants usually have synergistic toxicity impacts that elevate several-fold the mortality of organisms living in those environment (Rico-Martínez *et al.*, 2013). As crustacean species, shrimps are also more prone to acidic environment as their carapace are made from chitin or calcium carbonate that is vulnerable to dissolution as the ocean warms and decreases in pH (Asadi and Khoiruddin, 2017; Orr *et al.*, 2005).

The results showed that the toxicity of Pb increased over time which is shown in the probit analysis that Pb exposure at both pH 6.5 and 8.5 had very high correlation for 96-h exposure observation (R^2=0.958 and 0.935 respectively). At concentration of 562-1000 ppm, the Pb could kill all the test animals even at the earlier observation. However, at the concentration of 316 ppm, some of the shrimps could still withstand the toxicant particularly at the pH 8.5. The uptake of the metal via food chain, diffusion, and by the kinetics of reaction with transport proteins bounded the metal to the shrimp's lipid and brain (Chen *et al.*, 2014). Therefore, the test animals that suffered from this metal's exposure were unable to keep balance and swim. In longer periods of exposure and higher Pb concentrations, the shrimps became unconscious, comatose, and dead.

Furthermore, at 96-h observation, the LC_{50} value of pH 6.5 was significantly lower than that of at pH 8.5 (216.57 ppm and 259.84 ppm respectively). Therefore, the Pb metal in acidic environment is likely more toxic than in basic environment. In crustacean and other calcifying organisms, pH reduction can change the extracellular acid-base balance leading to disruption of the metabolic balance of the organisms, impacting biological processes of the organisms such as metabolism, fitness, calcification, and growth (Melzner *et al.*, 2009; Wu *et al.*, 2017). Study on *L. vannamei* exposed to chemical dispersant at pH 6.5 also shows that the LC_{50} value is more likely at the same level.

However at pH 8.5, Pb is much more toxic than that of chemical dispersant as the LC_{50} values of *L. vannamei* exposed to the oil dispersant at pH 8.5 is 553 mgL^{-1} (Asadi *et al.*, 2017). Study on Dusky Millions Fish, *Phalloceros caudimaculatus* exposed to lead nitrate showed that the LC_{50} is 15.5 ppm which is much lower than in whiteleg shrimps (Sanches-Filho *et al.*, 2017). However, Pb pollution both in acidic and basic environment is still practically non-toxic to the shrimps as the LC_{50} values were higher than 100 ppm (National Research Council (NRC), 2002).

Although, on the 96-h observation, the acidic environment slightly increased the toxicity of Pb on *L. vannamei,* the univariate analysis of variance of all the mortality data showed that pH and the interaction between pH and Pb did not statistically impact the mortality of the test animal as the *F*-stat was significantly lower than F-table. Meanwhile *F*-stat of Pb concentrations was much higher than the *F*-table, 87.13 and 2.21 for the *F*-stat and *F*-table respectively. Therefore, there were no synergistic toxicity effect of the combination of acidic or basic environment and Pb concentration in which those environments do not elevate the toxicity to the test animal several fold higher (Rico-Martínez *et al.*, 2013).

Conclusion

The research was intended to investigate the effect of pH to the toxicity of lead to whiteleg shrimps *L. vannamei*. It was clearly shown that Pb with both pH 6.5 and 8.5 have adverse impact to the post larvae *L. vannamei,* in which at the concentration of 1000 ppm, all the shrimps died even at the first 12 h observation. The 96-h LC_{50} values at pH 6.5 was significantly lower than that of at pH 8.5 (216.57 ppm and 259.84 ppm respectively). Although, the univariate analysis of variance of all mortality data from the 12 h to 96 h observations showed that Pb concentrations were the only sources of variation that had *F*-stat > F-table. It indicates that Pb concentrations were the only factors that induced the mortality, and the interaction of Pb and the pH does not cause synergistic toxicity to the whiteleg shrimps *L. vannamei*.

Acknowledgments

The publication of the research was supported by the dean of Faculty of Fisheries and Marine Science, Brawijaya University and the rector of Brawijaya University. The author acknowledges the technical and laboratory contributions from Ahmad Didin Khoiruddin and Risris Kemalarasi.

References

Asadi MA, Khoiruddin AD. 2017. pH effects in the acute toxicity study of the crude oil-WAF (water accommodated fraction) in the whiteleg shrimp, *Litopenaeus vannamei*. AACL Bioflux **10,** 1248–1256.

Asadi MA, Khoiruddin AD, Andrimida A. 2017. The influence of pH on oil dispersant toxicity to the whiteleg shrimp, *Litopenaeus vannamei*. J. Biodivers. Environ. Sci. **10,** 201–208.

Baltas H, Sirin M, Dalgic G, Bayrak EY, Akdeniz A. 2017. Assessment of metal concentrations (Cu, Zn, and Pb) in seawater, sediment and biota samples in the coastal area of Eastern Black Sea, Turkey. Mar. Pollut. Bull. **122,** 475–482.
https://doi.org/10.1016/j. marpolbul.2017.06.059

Chen K, Li E, Gan L, Wang X, Xu C, Lin H, Qin JG, Chen L. 2014. Growth and Lipid Metabolism of the Pacific White Shrimp *Litopenaeus vannamei* at Different Salinities. J. Shellfish Res **33,** 825–832. https://doi.org/10.2983/035.033.0317

Dall W, Hill BJ, Rothlisberg PC, Sharples DJ (Eds.). 1991. 9. Food and Feeding, in: Advances in Marine Biology. Academic Press pp. 315–332. https://doi.org/10.1016/S0065-2881(08)60175-3

Furtado PS, Fugimura MMS, Monserrat JM, Souza DM, Garcia LDO, Wasielesky W. 2015. Acute effects of extreme pH and its influences on the survival and biochemical biomarkers of juvenile White Shrimp, *Litopenaeus vannamei*. Mar. Freshw. Behav. Physiol **48,** 417–429.
https://doi.org/10. 1080/10236244.2015.1086539

Harrison RM. 2001. Pollution: Causes, Effects and Control: Edition 4. Royal Society of Chemistry, Washington DC.

Lee K-W, Shim WJ, Yim UH, Kang J-H. 2013. Acute and chronic toxicity study of the water accommodated fraction (WAF), chemically enhanced WAF (CEWAF) of crude oil and dispersant in the rock pool copepod T*igriopus japonicus*. Chemosphere **92,** 1161–1168.
https://doi.org/10.1016/j.chemosphere.

Mazzei V, Longo G, Brundo MV, Sinatra F, Copat C, Conti OG, Ferrante M. 2014. Bioaccumulation of cadmium and lead and its effects on hepatopancreas morphology in three terrestrial isopod crustacean species. Ecotoxicol. Environ. Saf. **110,** 269–279.
https://doi.org/10.1016/j.ecoenv.2014

Melzner F, Gutowska MA, Langenbuch M, Dupont S, Lucassen M, Thorndyke MC, Bleich M, Pörtner H-O. 2009. Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny?. Biogeosciences **6,** 2313–2331.
https://doi.org/ 10.5194/bg-6-2313-2009

Muñoz PN, Garbe-Schönberg C-D, Salamanca MA. 2004. Tracing the anthropogenic lead sources in coastal sediments of SE-Pacific (36° Lat. S) using stable lead isotopes. Mar. Pollut. Bull **48,** 688–697. https://doi.org/10.1016/j.marpolbul.2003.10.012

National Research Council (NRC). 2002. Oil in the sea III: Inputs, fates and effects. National Research Council, Washington DC.

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnanadesikan A, Gruber N, Ishida A, Joos F, Key RM, Lindsay K, Maier-Reimer E, Matear R, Monfray P, Mouchet A, Najjar RG, Plattner G-K, Rodgers KB, Sabine CL, Sarmiento JL, Schlitzer R, Slater RD, Totterdell IJ, Weirig M-F, Yamanaka Y, Yool A. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature **437,** 681–686.
https://doi.org/ 10.1038/nature04095

Rand G. 1995. Fundamentals of aquatic toxicology : effects, environmental fate, and risk assessment. Taylor & Franchis, Washington DC.

Rico-Martínez R, Snell TW, Shearer TL. 2013. Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A® to the *Brachionus plicatilis* species complex (Rotifera). Environ. Pollut **173,** 5–10.
https://doi.org/10.1016/j.envpol.2012.09.

Sanches-Filho PJ, Caldas JS, da Rosa NN, Pereira FOP. 2017. Toxicity test and Cd, Cr, Pb and Zn bioccumulation in *Phalloceros caudimaculatus*. Egypt. J. Basic Appl. Sci. **4,** 206–211.
https://doi. org/10.1016/j.ejbas.2017.06.001

Silva E, Viana ZCV, Onofre CRE, Korn MGA, Santos VLCS. 2016. Distribution of trace elements in tissues of shrimp species *Litopenaeus vannamei* (Boone, 1931) from Bahia, Brazil. Braz. J. Biol. **76,** 194–204.

Subramani PA, Michael RD. 2017. Chapter 4 - Prophylactic and Prevention Methods Against Diseases in Aquaculture A2 - Jeney, Galina, in: Fish Diseases. Academic Press pp. 81–117.
https://doi.org/10.1016/ B978-0-12-804564-0.00004-1

Sun X, Yang J, Zhang W, Zhu X, Hu Y, Yang D, Yuan X, Yu W, Dong J, Wang H, Li L, Kumar RV, Liang S. 2014. Lead acetate trihydrate precursor route to synthesize novel ultrafine lead oxide from spent lead acid battery pastes. J. Power Sources **269,** 565–576.
https://doi.org/10.1016/ j.jpowsour.2014.07.007

Szmytkiewicz A, Zalewska T. 2014. Sediment deposition and accumulation rates determined by sediment trap and 210Pb isotope methods in the Outer Puck Bay (Baltic Sea). Oceanologia **56,** 85–106.
https://doi.org/10.5697/oc.56-1.085

Valdes J, Calderon C. 2012. Metals content in sediments and benthic organisms of San Jorge Bay, Antofagasta, Chile. Revista de Biologia Marina Y Oceanografia **47,** 121-133**.**

Wu Q, Wang S, Chen X, Li P. 2017. Reproductive toxicity assessment of benzo[a]pyrene in the marine polychaete Perinereis nuntia. Chin. J. Oceanol. Limnol. **35,** 867–873.
https://doi.org/10.1007/ s00343-017-6024-6