TOXIC EFFECT OF MERCURONITRATE (HGNO3) ON VISCERA ORGANS OF RIVER FISH

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Abstract

Mercury waste is one of primary polutants found in the environment, either from industrial, hospital and household wastes, agricultural acitivities, or volcano eruptions. Mercury is a metal element that occurs naturally in the environment. Experiment carried out to assess mercurous salt on the development of freshwater fish, ie *Cyprinus carpio*, *Oreochromis niloticus*, *Pangasius hypophtalmus*, *Channa strata*, and *Clarias batrachus*. The results of experiments showed that sensitivity grade of each fish are quite difference in accordance with the ability of fish to detoxify mercurous salt absorbed to fish body. There are many similarities in the toxic effects of the various forms of mercury, but there are also significant differences. Toxicity test result showed that *Oreochromis niloticus* is very sensitive to this polutant with LC_{50} 0.99 ppm. The lowest sensitivity grade is found for *Cyprinus carpio*, LC_{50} 2.03 ppm. Respectively, sensitivity grades for each species are *Pangasius hypophtalmus* (LC_{50} 1.91 ppm), *Clarias batrachus* (LC_{50} 1.70 ppm), and *Channa strata* (LC_{50} 1.60 ppm).

Key word : Histopathological changes, organ vicera, freshwater fish ,mercury (Hg)

1. INTRODUCTION

Arinafril dan Müller (1996) defined a pollution as unnecessity Physical, chemistry and biology changing in aquatic ecosystems that harmed the source of life

One of pollutant that give negative impact to environment is mercury (Hg). Mercury waste is one of primary polutants found in the environment, either from industrial, hospital and household wastes and etc. The Residues of mercury can be founded in water, soil, plant tissue, animal and human body, as well as fossils. Currently, mercury can be found by fish consumming, dental amalgams and vaccines (Arinafril et al., 2001; Glickstein 2004; Vidal and Horne 2003). The Effect of mercury as a pollutant on marine life can be directly or indirectly, for example the reduction of water quality. The ability to accumulate mercury in the body can endanger the lives of marine biota and other biota such as the food chain. The use of some species of living organisms as bioindikator of environmental pollution has done a lot, both in vivo and in vitro studies, such as bacteria, lichens, fish, and others. Some of the pollutants have been studied in Palembang as shown in below Table 1 (Arinafril and Mueller 1996; Arinafril et al., 2001, Pena-Llopis, Fernando and Penya 2003; Wheelock et al., 2005, Dardenne et al.,2008).

Table 1. Pollutan, LC_{50} various species and organ

Pollutan	LC_{50} (mg L^{-1})	Species and organ	LT_{50} (hour)
Endrin	0,0006	Fish	96
BHC	0,790	Fitoplankton	48
Dieldrin	0,0008	Zooplankton	12
DDT	0,016	Intestine	96
Kadmium	0,01 – 10,0	Membran cell	24
Mercury	0,1 – 8,5	Bone	48
Black timber	0,3 – 4,5	Protozoa	124
Sianida	0,04		
Fenol	0,243		
Klorin bebas	0,03 – 0,2		

2. METHODOLOGY

This study used several Fish such as *channa striata*, *Oreochromis niloticus*, *Cyprinus carpio L*, *Clarias batrachus Pangasius hypophtalmus*. All fish were weighed and founded that the average weight of a fish is 1.5 - 2, - g. Preliminary trials carried out to obtain a concentration range that will be used for acute toxicity tests, After obtained the lethal concentration, the toxicity test conducted. Mercurous salt is made into standard solutions, with 5 levels of concentration of each type of fish with 6 replications. Each type of fish used different concentration between 0.1 ppm and 1.4 ppm.

The first prosedure is manufacturing solution, this solution will be counted in weight per volume with units of grams / liter counts. The experimental design used is Complete Randomized Design (CRD) by five treatments and six replications. The treatment under study is the use of salt mercorous(HgNO3) with 5 levels of concentration and one control.

The Observations toward the fish given salt mercurous with 5 grades of concentration,will be compared with the control treatment, which is healthy and did not change during the experiment .

After the trial ended,five kinds of fish that have been poisoned by salt mercurous from all concentration are dissected and observed their morphology, and than compared with the control treatment.In order to make the similarity in the observation, the fish used are in the same of size and age.

The study is conducted by using probit analysis through SPSS Windows Version 11.0 to get the effect of several concentrations of mercury toward several fish studied (Lethal Concentration) and the length of time until the fish dies (Lethal Time).

3. RESULTS AND DISCUSSION

The results showed that there is a relationship between increase of concentration and mortality of various species of freshwater fish, as shown in the below table

Table 2. The relationship between concentration and mortality

Consen-trate	Mortality *cyprinus carpio*	Mortality *Pangasius hypophtalmus*	Mortality *Oreochro-mis niloticus*	Mortality *channa striata,*	Mortality *Clarias batrachus*
0	0	0	0	0	0
1	2	0	2	5	3
2	3	0	3	6	4

3	4	1	4	6	5
4	6	4	5	8	6
5	8	6	7	10	9
Uji- F	41.035**	26.299**	37.239**	51,176**	29,345**
LC_{50}	2.03	1,91	0,99	1,60	1,70

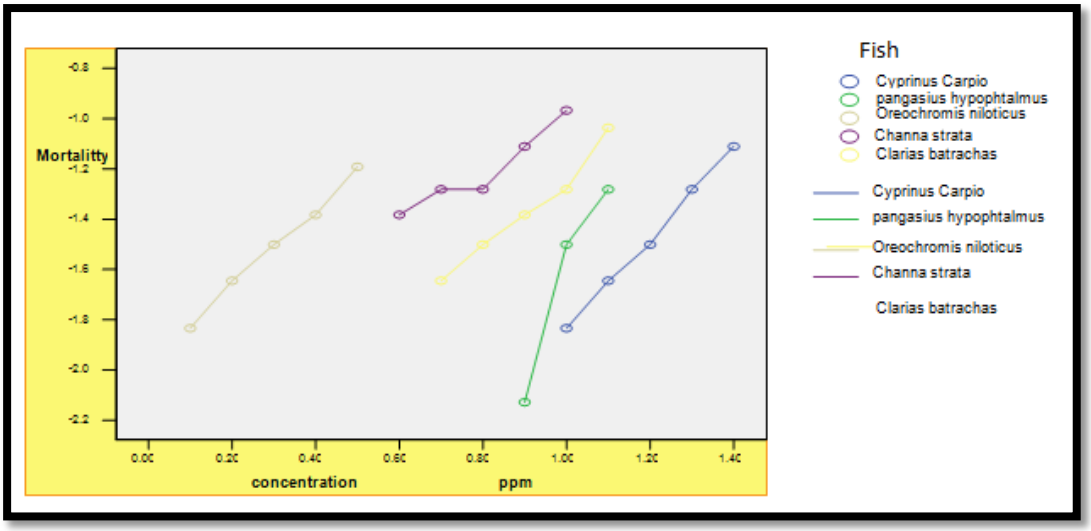
Explanation:

Concentrate	***cyprinus carpio***	***Pangasius hypophtalmus***	***Oreochromis niloticus***	***channa striata***	**Mortality *Clarias batrachus***
1	1 ppm	0,7 ppm	0,1 ppm	0,6 ppm	0,7 ppm
2	1,1 ppm	0,8 ppm	0,2 ppm	0,7 ppm	0,8 ppm
3	1,2 ppm	0,9 ppm	0,3 ppm	0,8 ppm	0,9 ppm
4	1,3 ppm	1 ppm	0,4 ppm	0,9 ppm	1 ppm
5	1,4 ppm	1,1 ppm	0,5 ppm	1 ppm	1,1 ppm

Various studies have been conducted to determine the LC value of various pollutants on aquatic organisms. According to Vidal and Horne (2003), LC50 of marine worms Sparganophilus pearsei is 0.22 mg / l or 0.22 ppm. Devlin (2006) showed acute toxicity of embryos Pimephales promelas fish has value of LC 221 ug / l or 0.221 ppm for 24 hours a day and 39 mg / l or 0.039 ppm for 96 hours. Glickstein (2004) Stated that the LC crab Cancer magister 6,6 µg/l or 0,0066 ppm for 96 hours, but LC^{50} of Crassostrea gigas fish is 5,7 µg/l or 0,0057 ppm for 24 hours.

According to Verma, Jain dan Tonk (2002) SLC^{50} after 30 days contaminated by Notopterus notopterus is 0,088 µg/l or 0,000088 ppm.

Probit graphic of whole fish examined will be shown in the below Figure 1.



On treatment at concentration 1 ppm is seen that the *Cryprinus Carpio* gills redden, and discharge blood. Its movement become slow and two of them are dead and floating on the water.

At concentration 1.1 ppm, *Cryprinus Carpio* gills gills redden, and discharge blood. Its movement become slow and two of them are dead and floating on the water.

At concentration 1.2 ppm, *Cryprinus Carpio* gills gills redden, and discharge blood Its motion become slow in which the body discharge mucus and the position of the mouth at the top. At this concentration four of them are dead. At concentration 1.3 ppm the fish swimming sideways, gasping for air, the fish began to adapt, swimming tilted back, swimming upside down and five of them are died. At Concentration 1.4 ppm shown that the fish gills being to bleed, they swim on the bottom of media in dying condition. At this concentration there are six fish died in the surface of water.

When compared with the control situation of the *Cryprinus Carpio* surgical operation on its body i.e good red intestine,bile and gills. so the incision were subjected to be salt mercurous treatment. At concentration 1 ppm the intestine still red,buble and bile are intact but produced mucus. At the concentration 1,1 ppm the intestine still red , buble and bile are intact. On the concentration 1,2 ppm the gills get bleeding but intestine is pale. At the concentration 1,3 ppm intestine pale. At the 1,4 ppm concentration shown that buble and bile broken.

At the treatment 0,7 ppm concentrate showed two *pangasius hypophtamus* become pale, motionless in the bottom of media, one of the gills opened. It begin to swim but can not reach the surface of water. At the concentration 0,8 ppm two *pangasius hypophtamus* became pale, motionless in the mediun bottom, two *Pangasius hypophtamus* gills opened.It begin to swim but can not reach the surface of water.At the concentration 0,9 ppm two catfish became pale, the gills cover of two fish opened, the gillls cover of third fish opened, swim on the surface, its glomy,a *pangasius hypophtamus* begin to faint, gills become wider and opened and the last swim un order. At the 1 ppm concentration, two *pangasius hypophtamus* became pale, passive on the bottom, , these three fish are pale. At the concentration 1,1 ppm two fish are pale, passive in the bottom, blood gather on the head of two fish, these three fish are pale.

Compared with the control situation, the condition of surgical *pangasius hypophtamus* on its body i.e health red intestine,bile and gills. so the incision were subjected to be salt mercurous treatment with concentration 0,7 ppm so *pangasius hypophtamus* body liquid become reddish brown,intestine reddish yellow (close to control situation). At the concentration 0,8 ppm, the gills begin to bleed, intestine turn yellow. At the concentration 0,9 ppm, the gills bleeding and intestine turn yellow. At the concentration 1 ppm, the gills bleeding, bile broken, intestine redden and at the concentration 1.1 ppm the intestine become redish black.

On the treatment 0,1 ppm, two *Oreochromis niloticus* supple in the bottom, one Oreochromis niloticus drew bood through the gills, two faint, one die, and they are die at the end. At concentration 0,2 ppm *Oreochromis niloticus* supple in the bottom, one is dying, two fish supple in the bottom, one *Oreochromis niloticus* gills bloody, those three fish are faint. The first *Oreochromis niloticus* die,second *Oreochromis niloticus* die, third aslo died. At the concentration 0,3 ppm, seen that one *Oreochromis niloticus* is dying, two *Oreochromis niloticus* swim to the surface, one *Oreochromis niloticus* swims on the bottom ,two *Oreochromis niloticus* are dying , faint, two *Oreochromis niloticus* die, one *Oreochromis niloticus* floating , one *Oreochromis niloticus* sink, third *Oreochromis niloticus* dead. At the consentration 0,4 ppm the gills of three fish opened, faint, two *Oreochromis niloticus* die, third *Oreochromis niloticus* faint and than died. At the consentration 0,5 ppm the gills of three *Oreochromis niloticus* opened. 1 *Oreochromis niloticus* faint, one *Oreochromis niloticus* bloody in its gills, weak and swim on the bottom, one supple on the surface, after that these three fish became supple and finally dead .

Compared with the control situation, the condition of surgical incision of *Oreochromis niloticus* on its body i.e red intestine, bile and gills in the good condition, the result of an incision in the fish *Oreochromis niloticus* treated with mercurous salt at a concentration 0.1 ppm i.e the body fluid become red. At the Concentration 0.2 ppm body fluid is still red , gills bleeding, intestines turn blue and drew mucus. At the Concentration 0.3 ppm, the body fluid turn red, intestine pale and mouth drew mucus. At Concentration 0.4 ppm in which body fluids

become pale, intestine turn yellow, mouth drew mucus. At te concentration 0.5 ppm, the gills bleeding, body fluids become pale, intestine became black, and mouth produced mocus.

On treatment with a concentration 0.6 ppm is seen 2 (two) *channa strata* turned loose to swim, it seem that the fish remove its feces, two *channa strata* swim by the head above, all *channa strata* get supple, 4 fish are dead. At the concentration 0.7 ppm seem that 2 *channa strata* limp, begin to remove its feces, gills begin to bleed, drew its feces dirt and 3 *channa strata* died. At the Concentration 0.8 ppm is seen that the fish getting restless, fish begin to drew the buble, the fish becomes weak, redden gills, the fish started jumping up and down, the fish limp by swimming at the bottom, slow motion, the fish swim sloping in the surface. At the concentration 0.9 ppm seem that the fish getting restless, fish bubbles, the fish began to supple, scales fish begin to exfoliate, gills redden, started jumping up and down because lack of oxygen, fish became weak and swim on the bottom of media, slow movements, the fish swim slantly on the surface, fish become weak and swim upside down on the surface, finally they are dead. At the concentration,1 ppm seen that the blood of two fish gather on the head. The fish is active but on the bottom, the gills redden and started jumping up and down because lack of oxygen, the fish swim sloping on the surface, and finally dead.

When compared with the control situation of the surgical incision of the *channa strata* on its body that is good red intestine, bile and gill, so the *channa strata* incision treated with mercurous salt with 0.6 ppm concentration the result intestine became pale. At the cooncentration0.7 ppm intestine became pale, mouth discharge mucus. At the concentration 0.8 ppm intestine became pale, mouth discharge mucus. Concentration of 0.9 ppm body fluids become pale, intestine turn yellow, mouth produced mucus. At the 1 ppm concentration, the gills get bleeding, body fluids become pale, and intestine became yellow and drew mucus from the mouth.

On the treatment with a concentration 0.7 ppm is seen the behavior of *clarias batrachas*. Two *clarias batrachas* swimming weakly in the bottom,one *clarias batrachas* drew blood through its gills and than die. At the consentration 0,8 ppm is seen that *clarias batrachas* supple on the bottom, bleeding on its gills, andd some of them supple and dead. At the concentration 0,9 ppm shows some of *clarias batrachas* are dying, supple and floating on the water and also sink on the bottom. At the concentration 1 ppm is seen that some *clarias batrachas* gills opened, some fish are faint, sink and floating on the surface of water.At the concentration 1.1 ppm some fish's gills opened, there is a bleeding gills, supple and swimming on the bottom, and eventually died..

Compared with the control state of the surgical incision of *clarias batrachas* fish on its body i.e white intestines, dark red fluid in the good condition, the incision of lele is treated with mercurous salt with 0.7 ppm concentration. The result, body liquid become pale and intestine turn yellow. At the concentration 0,8 ppm body liquid paler and intestine became yellow and mouth produced mucus. At the concentration 1 ppm, the body fluids are pale, intestine turn yellow, mouth discharge mucus. At the concentration 1.1 ppm made the liquid body paler, intestine turn yellow, and mouth produced mucus.

Metals (Hg) are very reactive toward sulfur and nitrogen ligands, so that the metallic bond is very important for normal function of metaloenzim and also to metabolism of cell . Here, enzyme plays an important role in the gills of *Clarias batrachu*s.i.e the carbonic anhydrase enzyme and transport ATP ase.Carbonic anhydrase is an enzymes containing Zn and has function to hydrolyze CO_2 into carbonic acid. If the bond of Zn is replaced with other metal, the enzyme carbonic anhydrase function will be decreased.

In addition to these biochemical disturbance, changing in morphological structure of the gills of *clarias batrachas* also occur. *clarias batrachas* will get hypoxia (due to difficulties in taking oxygen from the water), so that thickening of the gill epithelial cells happened and make the fish uncapable to swim. The toxicities of heavy metals that harm the gills and other external

tissue In addition to these biochemical disturbance, changing in morphological structure of the gills of *clarias batrachas* also occur. *clarias batrachas* will get hypoxia (due to difficulties in taking oxygen from the water), so that thickening of the gill epithelial cells happened and make the fish uncapable to swim. The toxicities of heavy metals that harm the gills and other external tissue structure are able to make the fish dead due to anoxemia process, i.e the inhibition of the respiratory function of gill circulation and excretion. Heavy metal elements that have influence on the gills are tin, zinc, iron, copper.

Kidney of *clarias batrachas* important for filtration and execrete the materials that are not needed by its body,such as heavy metals. This made the kidneys broke by the metal toxic. There was a precipitate in tubular lumen, and the damage is higher on the toxicity of Hg (Delamare and Truchet, 1984). The difference of toxicity degree of Heavy metal toward types of marine life can be shown by experiments conducted by Schweiger toward some types of fish (such as *trout* and *carp*) which is shown the difference sensitivity of that fish. Based on this experiment, it can be proved that the differences of sensivity has close relationships to differences of the activity of fish.The degree of toxicity also has relation with *respiratory flow* of each organism, that is the higher the respiratory flow, the toxicity of heavy metals will increases. Similarly, dissolved oxygen in low levels made the fish pumping the water through its gills more. more pumping water through the gills make *respiratory flow* increase and the result more toxins are absorbed into the body through the gills.In addition there are several ions from various heavy metals that synergize or antogonistic to each other, e.g Cd sinergyze with Mg.

According to Herawati (1980) mercury able to agglomerate mucus on the gills and damaged tissue of the gills and made them die. The heavy metals enter to the body of aquatic organism through three ways e.g through food, gills and diffusion through the skin(Fischer, Frederiksson, and Eriksson 2008). Gills is the important part of a fish because Gill surface more than 90% of the total area of the body, so that so that the entry of heavy metals in the gills can cause poisoning, it is caused by the reaction of metal cation with other fraction of the gills mucus. This condition made the metabolism of the gills disturbed, the production of mucus more than usual, so that the accumulation of mucus happened. This case will retard the expression of gills and finally caused the death (Sudarmadi 1993).

One of the changes occur due to dumping of waste into water is reduction of disolved oxygen levels. Mason (1980) stated that oxygen is the important things in the respitory process and the essential component for fish and other organisme metabolism. The organic compound in the water will be broke by purid organism. The occurance of this process needs dissolved oxyggen in the water(Duffus 1980). The toxic compounds contained in the waste also influences the metabolism processes in the body of fish, damage the intesine tissue and kidney function. These toxic compounds also affect blood and other organs. Besides the toxic compounds and heavy metals can inhibit the metabolism of serum proteins (Dardenne et al., 2008). Dardenne (2008) also stated that toxic compunds and heavy metals are able to obstruct the metabolism of protein serum (Dardenne *et al*. 2008).

Exposuring heavy metal cadmium to the fish *Pleuronectes flesus* made hematocrit value hemoglobin and the grade of red blood cell decreased, so that it caused anemia. Anemia is the situation of the body in which the plasma volume increase due to the balance system of fish body affected, clearly the causing of anemia is the decrease of blood cell production speed or the damage of blood cell rapidly(Larsson *et al*. 1976). The treatment of heavy metals to freshwater fish also causes a decrease of red blood level, hemoglobin and hematocrit values.(McDermott 2008).

The damage of Ecosystem that caused by heavy metal pollution, commonly occur in aquatic ecosystems. This occurs because of there are heavy metals that be toxic for the aquatic

organisms. So that, the most sensitive organisms will get damage and for those who unable to survive will destroyed, and the result the food chain and aquatic ecosystem will damage.

4. CONCLUSION

Based on the chart of kinds of fish, we are able to shows the results analysis of LC_{50} probit, the higher of giving concentration of salt mercurous (HgNo3)so the mortality rate of five species of freshwater fish are more.

LC value of *Cryprinus Carpio* is 2.02696 ppm it has pale intestine, bubbling and broken bile , mucous discharge and gills bleeding as the characteristics . LC value of *pangasius hypophtamus* is 1.910478 ppm characterized by dark red fluid, blackish red, gill bleeding and bile broke.. LC value of *Oreochromis niloticus* is 0.99912 ppm is characterized by red-colored fluid, bleeding gills, intestine pale, mouth produced mucus. The value of*Oreochromis niloticus* fish is LC 1.596174 ppm is characterized by pale intestine, mouth dischrage mucus and gills bleeding. LC value of *clarias batrachas* is 1.703648 ppm with a characteristic- body fluids is paler,intestine became yellow and mouth discharge mucus.

REFERENCES

Arinafril and P. Müller. 2001. Environmental pollution associated with the presence of pesticide in water. Aquat. Toxicol. 5 (2) : 31 – 36.

Arinafril, J. Krüger, J. Dittmann, A. Schäfer and P. Müller. 2001. Chemical hazardous substances in water and water risk assessment. Proceeding of National Conference on Water, Land and Food. Research Center for Water and Land Management, Sriwijaya University, Palembang, Indonesia. p. A 4.1 – 4.7.

Arinafril and P. Müller. 1996. Zur Bestimmung der Konzentration von Metallen aus Musi-Fluss, Palembang, Indonesia. Reson. 1 (2) : 28 – 32.

Arinafril. 1993. Rückstandanalystische Untersuchung von chlorierten Kohlenwasserstoffen in Rotaugen (*Rutilus rutilus*). Biogeog. 13 : 21 – 24.

Dardenne, F., S.V. Dongen, I. Nobels, R. Smolders,W.D. Coen and R. Blust. 2008. Mode of Action Clustering of Chemicals and Environmental Samples on the Bases of Bacterial Stress Gene Inductions. Toxicol. Sci. 101(2) : 206–214.

Duffus, H. J. 1980. Environment Toxicology. Department of Brewing and Biological Science. Hariot-Watt. University Edinburgh, Scotland.

Fischer, C., A. Fredriksson and P. Eriksson. 2008. Coexposure of Neonatal Mice to a Flame Retardant PBDE 99 (2,2#,4,4#,5-Pentabromodiphenyl Ether) and Methyl Mercury Enhances Developmental Neurotoxic Defects. Toxicol. Sci. 101 (2): 275 – 285.

Forstner, U. and G.T.W. Wittman. 1983. Metal Pollution in the Aquatic Environment. Second Edition. Springer Verlag, Heidelberg.

Glickstein, N. 2004. Acute toxicity of Mercury and Selenium to *Crassostrea gigas* Embryos and C*ancer magister* Larvae. Mar. Biol. 49 (2) : 113 – 117.

Larson, A, B.E. Bengston and O. Svaberg. 1976. Effect of Cadmium for Hematology and Biochemistry on Fish. Cambridge University Press, London.

McDermott, C., A. Allshire,F. van Pelt and J. J. A. Heffron. 2008. In Vitro Exposure of Jurkat T-Cells to Industrially Important Organic Solvents in Binary Combination: Interaction Analysis. Toxicol. Sci. 101(2) : 263–274.

Pena-LIopis, S., M.D. Ferrando and J.B Penya. 2003. Fish tolerance to Organo-phosphate-Induced Oxidative Stress is Dependent on the Glutathione Metabolism and Enhanced by *N*-Acetylcysteine. Aquat. Toxicol. 65 : 337-360

Sudarmadi. 1993. Toksiologi Limbah Pabrik Kulit terhadap *Cyprinus Carpio L*. dan Kerusakan Insang. Jurnal Lingkungan dan Pembangunan 13 (4) : 247-260.

Vidal, D.E. and A.J. Horne. 2003. Mercury Toxicity in the Aquatic Oligochaete *Sparganophilus pearsei:* I. Variation Resistance Among Population. Arch. Environ. Contam. Toxicol. 45(2) : 184 – 189.

Wheelock. C.E. J.L.Miller, B.M. Phillips, S.J.Gee, R.S. Tjeerdema and B.D. Hammock. (2005). Influence of Container Adsorption Upon Observed Pyretroid Toxicity to *Ceriodaphnia dubia* and *Hyalella azteca*. Aquat. Toxicol. 74 : 47-52.

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