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Acute Toxicity of Mercury to Three Freshwater Organisms Species in Malaysia

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ABSTRACT

Background: Mercury raise global concern due to the toxicity, potential to enter the food chains and harmed caused. In this study, three freshwater organisms were used to evaluate mercury toxicity level. Two fishes namely *Poecilia reticulata* (guppy) (Poeciliidae), *Rasbora Sumatrana* (Cyprinidae) and midge larvae *Chironomus javanus* (Chironomidae) were exposed for duration of 4-day (96h) period in laboratory conditions to a range of mercury (Hg) concentrations. Mortality was assessed and median lethal times (LT_{50}) and concentrations (LC_{50}) were calculated. **Objective**: To determine the acute toxicity of Hg concentration on *Poecilia reticula, Rasbora sumatrana* and *Chironomus javanus* in laboratory. **Results**: The LT_{50} and LC_{50} increased with the decreased in mean exposure concentrations and times, respectively. LC_{50}s for 24, 48 and 96h for *Poecilia reticulata* were 342.3, 179.2 and 95.8 µg/L, *Rasbora Sumatrana* were 156.7, 134.4 and 93.8 µg/L and *Chironomus javanus* were 222.9, 82.2 and 67.7 µg/L, respectively. **Conclusion**: Results indicated that *Chironomus javanus* was the most sensitive to mercury compared to *Poecilia reticulata* and *Rasbora Sumatrana* in order of *Chironomus javanus* > *Rasbora sumatrana* > *Poecilia reticulata*

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INTRODUCTION

Mercury is known as a widespread and persistent pollutant occurring in a variety of forms in freshwater and marine ecosystems (Satoh, 2000). Also, mercuric compounds are very toxic and the toxicity of mercury has been known since the beginning of the 16th century (Ramalingam *et al*., 2001). Water pollution by mercury (Hg) is an issue that raises great concern due to its high toxicity, resistant in the environment. Mercury is also a very mobile metal due to its high volatilization. Due to this characteristic, mercury able to be transported across wide area from it source and thus pose global concern. However, in spite of imposed worldwide legislation urging for zero mercury discharges (OSPAR), anthropogenic mercury emissions in Asia, especially that in China, are likely to increase significantly in the next decades (Lin *et al*., 2012). Therefore, Malaysia as one of developing country in Asia is not exceptional from mercury threat with concern of the mercury effect in aquatic ecosystem.

This element is classified as one of the most toxic metals, which are introduced into the natural environment by human interferences and disturbance (Buhl, 1997). Contamination and accumulation of toxic metals by an aquatic organisms has long since been a major environmental issues and is still growing at an alarming rate (Martín-Díaz *et al*., 2005). Exposure of aquatic organisms to mercury can cause a multiplicity of adverse effects, arising from the binding of mercury ions to functional groups in proteins, namely sulfhydryl, phosphoryl, carboxyl, amide, and amine groups. Such interactions may cause protein precipitation, enzyme inhibition and corrosive action (Broussard *et al*., 2002). In evaluating environmental condition, benthic macroinvertebrates are commonly used due to their abundance, distribution and ranges of sensitivity (Buss *et al*., 2015). Chironomus spp is one of the most ubiquitous benthic groups that can be found in both lotic and lentic environments and playing an important role as high protein food source for fish (Armitage *et al*. 1995). The fish abele to accumulate substantial amount of Hg in their tissues, and thus can represent a major dietary source of this heavy metal for humans. Hence, fish are the single largest sources of Hg for humans. Prevalence of Hg in the environment leads to biomagnification in the food chain (Pandey, 2012). Thus toxicity testing is an essential tool for assessing the effect and fate of toxicants in our aquatic ecosystems. Also as a tool to

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identify suitable organisms for being used as bioindicator and to derive water quality standards for chemicals (Adam, 2010).

The goal of this study is to determine the acute toxicity of Hg to *Poecilia reticulata*, *Rasbora sumatrana* and *Chironomus javanus* in laboratory. Metals research in Malaysia, especially using organisms as bioindicator is still scarce. Because of that, it is crucial to conduct research using local organisms in toxicity testing for the determination of the organism's sensitivity toward metal and also to derive a permissible limit for Malaysian's water quality standard in order to protect aquatic ecosystems (Shuhaimi-Othman *et al*., 2012).

Methodology:

Rasbora sumatrana were purchased from aquarium shops in Bangi, Selangor, *Poecilia reticulata* were collected from the drain at National University of Malaysia while *Chironomus javanus* were collected from a drain outside Institut Kemahiran Belia Negara Dusun Tua, Hulu Langat, Selangor. Prior to toxicity testing, the organisms were acclimatized for one week under laboratory conditions which is 27 - 30˚C room temperature with 12 h light and 12 h darkness in different stocking tanks using dechlorinated tap water filtered by several layers of sand and activated carbon, and aerated through an air stone. During acclimation, organisms were fed with commercial fish food pellet (Super-Gold Tropical Fish Food). Fish was fed daily but during the final acclimation organism was not fed to avoid the interference. The stock solution (1000 mg/L) of mercury (Hg) was prepared from analytical grade salts mercury chloride $HgCl_2$ (Merck, Germany). The stock solution was prepared with deionized water in 1L volumetric flasks. For preliminary test, organisms were exposed to 5 different concentrations of 0.1, 1.0, 10, 100 and 1000 mgL^{-1}. Acute Hg toxicity experiments were performed for 4-day period using organisms (*P. reticulata* approximately 2.5 – 3.5 cm body length, wet weight 2.0 -2.5 g, *R. sumatrana* 5.0 – 6.0 cm body length, wet weight 3.5 – 5.0 g, *C. javanus* approximately 0.6 – 1.1 cm) obtained from stocking tanks. Based from the preliminary test, five concentrations of mercury were chosen (0.1, 0.18, 0.32, 0.56 and 1.0 mg/l). Metal solutions were prepared by diluting of stock solution with dechlorinated tap water to desired concentrations. A control with dechlorinated tap water was used. The tests were carried out under static conditions with the renewal of the exposure solution every two days. Four replicates of control and metal-treated groups each consisted of five randomly allocated organisms in 500 mL beaker containing 350 ml of appropriate solutions.

All controls resulted in low mortalities, fewer than 5%, which indicated the acceptability of the experiments. A total of 20 replicate per treatment were used in the experiment and total of 110 for each species were employed in the investigation. Water samples were taken before and immediately after each solution renewal for metal analysis. Each sample of Hg solution by 2% (HCL 37%) and measured by inductive couple plasma mass spectrometry (Model ELAN 9000 Perkin Elmer ICP-MS, USA). Mortality was recorded every 3 hour for the first 2 days and then at 4 hour until 4 days. The mortality was determined by failure to respond to gentle physical stimulation and any dead organisms were removed immediately. Median times of death (LT_{50}) and lethal concentrations (LC_{50}) for the fishes exposed to Hg were calculated using measured metal concentrations. FORTRAN programs based on the methods of (Litchfield, 1949) and (Litchfield and Wilcoxon, 1949) were used to compute and compare the LT_{50} and LC_{50}. Data were analyzed using both time / response (TR) and concentration / response (CR) methods by plotting cumulative percentage mortality against time and concentration on logarithmic probit paper.

RESULTS AND DISCUSSION

In all data analyses, the actual rather than nominal Hg was used and shown in Table 1. The mean water quality parameters measured during the test were 26.1 ± 0.2˚C for water temperature, 7.1 ± 0.1 for pH, 470 ± 0.8 μScm^{-1} for conductivity, 6.8 ± 0.2 mg/L for dissolved oxygen, and 29.8 ± 1.2 mg/L as $CaCO_3$ for total hardness (Mg^{2+} and Ca^{2+}). Ninety percent of control organisms maintained in dechlorinated water survived throughout the experiment. The median lethal times (LT_{50}) and concentrations (LC_{50}) increased with the decrease in mean exposure concentrations and times, respectively for both species of fish and Chironomidae (Table 1 and Table 2).

Data for median lethal times (LT_{50}) were plotted against concentrations and median lethal concentrations (LC_{50}) were plotted against time of exposure (Figure 2) showed that *C.javanus* was more sensitive to the exposure of Hg compared to *P. reticula* and *R. sumatrana*. It was also showed that *R. sumatrana* was more sensitive to all eight metals compared to *P. reticulata* (Shuhaimi-Othman *et al*., 2013a). This indicates that different organisms have different sensitivity levels to metals toxicity. The differences are due to the natural biological variations reflecting the population genetic make-up and the individual condition.

The variation is normally small for organisms of the same species, age and health and generally greater between species (Rand *et al*., 1995). As stated by Boening (2000), organic mercury is retained in the tissues of aquatic organisms over a longer period than inorganic mercury. Several factors affect the susceptibility of aquatic organisms to mercury. These include the life stage (the larval stage is highly sensitive), the development of tolerance, water temperature and water hardness.

The variations in sensitivity of aquatic species among invertebrates and vertebrates to toxic chemicals observed that the LC_{50} values was small for narcotic chemical (eg. Acetone and benzene) and much larger for chemicals with a specific mode of action such as metals (Hoekstra *et al.*, 1994). The susceptibility of fish species to a particular heavy metal is a very important factor for LC_{50} levels. Fish that is highly susceptible to the toxicity of one metal may be less or even nonsusceptible to the toxicity of another metal at the same level of that metal in the ecosystem. Also, the metal which is highly toxic to a fish species at low concentration may be less or even nontoxic to other species at the same or even higher concentration (Hedayati *et al.*, 2010).

Study showed that *C. javanus* and *R. sumatrana* were the most sensitive than *P. reticulata* to different exposure of Cu, Cd, Al and Mn (Shuhaimi-Othman *et al.*, 2013b). The rank order of toxicity of metals will vary between organisms, and the factors that affect the rate of uptake of metals affect the toxicity of metals. Toxicity ensues once the threshold of metal availability has been passed, indicating that the rate of uptake exceeds both the rate of excretion and detoxification (Louma and Rainbow, 2008). In this study, *P. reticulata* showed the highest LC_{50} 96h (95.8 µgL^{-1}) compared to *R. sumatrana* (93.8 µg/L) and *C. javanus* (67.7 µg/L). Higher LC_{50} values are less toxic because greater concentrations are required to produce 50% mortality in animals. As discussed by World Health Organization, The 96h LC_{50} values for freshwater fish ranges between 33 and 400 µg/L, while the 96h LC_{50} is generally higher for marine fishes (Boening, 2000).

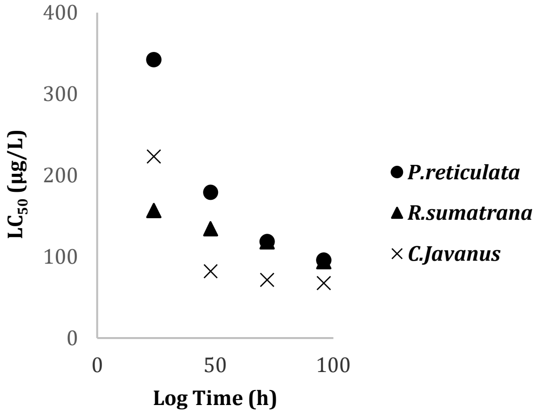

Fig. 1: The relationship between median lethal concentration (LC_{50}) and exposure time (h) *for P. reticulata, R. sumatrana* and *C. javanus.*

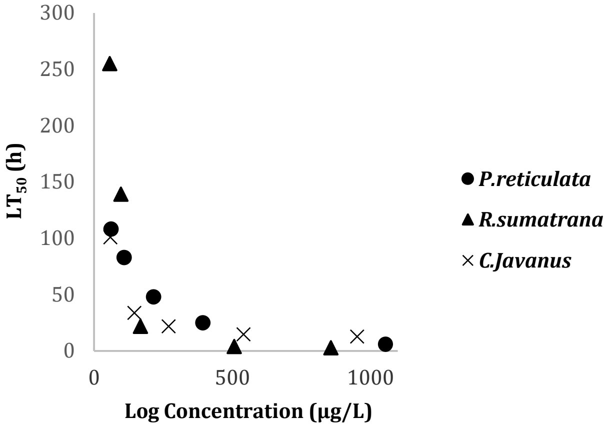

Fig. 2: The between median lethal concentration (LT_{50}) and different concentrations (µg/L) for *P.reticulata, R.sumatrana* and *C.javanus.*

Table 1: Median lethal times (LT_{50}) for *P. reticulata*, *R. sumatrana* and *C. javanus* exposed to different concentrations of Hg.

Nominal Concentration (µg/L)	Measured Concentration (µg/L)	LT_{50} (h)	95% confidence limits	Nominal Concentration (µg/L)	Measured Concentration (µg/L)	LT_{50} (h)	95% confidence limits
P.reticulata				*R.sumatrana*			
100	61.4	108	52 – 223	100	56.3	255	50 – 1303
180	108.4	83	50 – 137	180	97.1	139	35 – 548
320	214.8	48	29 – 77	320	166.5	22	11 – 41
560	392.6	25	17 – 37	560	508.0	4	3 – 5
1000	1053.5	6	4 - 8	1000	857.3	3	2 - 4

Nominal Concentration (µg/L)	Measured Concentration (µg/L)	LT_{50} (h)	95% confidence limits
C.javanus			
100	58.8	101	42 – 245
180	146.1	33	26 – 42
320	269.3	22	17 – 29
560	540.9	15	11 – 21
1000	950.8	13	9 - 18

Table 2: Median lethal times (LC_{50}) for *P.reticulata*, *R.sumatrana* and *C.javanus* exposed to different concentrations of Hg.

Time (h)	LC_{50} (µg/L)	95% confidence limits	Time (h)	LC_{50} (µg/L)	95% confidence limits	Time (h)	LC_{50} (µg/L)	95% confidence limits
P.reticulata			*R.sumatrana*			*C.javanus*		
24	342.3	261 – 444	24	156.7	126 – 219	24	222.9	158 – 330
48	179.2	117 – 250	48	134.4	92 – 187	48	82.2	51 – 111
72	118.7	68 – 173	72	118.1	76 – 150	72	71.3	47 – 94
96	95.8	51 - 139	96	93.8	64 - 120	96	67.7	-

Table 3: Comparison of LC50 96h in freshwater fishes in this study with other research.

Species	Water hardness (mg/L)	Temperature (˚C)	LC_{50} (µg/L)	Reference
Poecilia reticulata	29.8	26.1	95.8	This study
Rasbora sumatrana	29.8	26.1	93.8	This study
Poecilia reticulata	na	23	200	(Slabbert and Venter, 1999)
Oreochromis niloticus	24.6	24	220	(Ishikawa *et al*., 2007)
Rutilus rutilus	na	na	350	(Hedayati *et al*., 2012)
Varicorhinus barbatus	140 - 160	24-26°C	168	(Shyong and Chen, 2000)
Zacco barbata	140 - 160	24-26°C	161	(Shyong and Chen, 2000)

Table 4: Comparison of LC50 96h in Chironomidae in this study with other research.

Species	Water hardness (mg/L)	Temperature (˚C)	EndPoint (hour)	LC_{50} (µg/L)	Reference
Chironomus javanus	29.8	26	96	67.7	This study
Chironomus riparius	50	20	96	100	(Boening, 2000)
Chironomus sp	50	20	96	20	(Boening, 2000)
Chironomus riparius	50	20	96	547	(Boening, 2000)
Chironomus riparius	na	na	48	1580	(Rodrigues *et al*., 2013)

In comparison with other researches, *P. reticulata* in the present study showed high sensitivity to mercury which is LC_{50} 96h 95.8 µg/L while Slabbert JL & Venter EA showed LC50 96h 200 µg/L (1999). This is probably due to the test was conducted in hard water compared to present study which used soft water (29.8 mg/L $CaCO_3$) as this has known to give effect as the water hardness decreased the toxicity of mercury increased (Terzi and Verep, 2011). It also supported when Rodgers (1982) found that the uptake of methyl mercury by rainbow trout was less in hard water (385 mg/L $CaCO_3$) than in soft water (30 mg/L $CaCO_3$) and suggested that increased mercury uptake efficiency in soft water may partially explain elevated mercury levels observed in fish from lake of low alkalinity and pH.

Based on the observation by World Health Organization on various both freshwater and marine fish species showed that age of fish are correlates with concentration of mercury in the tissues they accumulated throughout the life span. In some species, males have been found to have tendency accumulate higher mercury levels than females of equal age (Boening, 2000). Different invertebrate groups provide different mode of action and / or mechanism of self-defense even though exposed to the same conditions (medium, temperature, photoperiod, absence of food). Thus, it is relevant to study mercury toxicity to a wide variety of organisms since they might be affected differently (Rodrigues *et al*., 2013). Because of the lack of available data on the effects of Hg on the respective LC_{50} values of all studied species in Malaysia, the results of the present study have not been compared with those of other studies and discussed accordingly. However, some data have been provided following various studies (Table 3 and Table 4).

Conclusion:

It was concluded that *C.javanus* was the most sensitive to the Hg compared to *R. sumatrana* and *P. reticulata* in order of *C. javanus* > *R. sumatrana* > *P. reticulata*. All of these observations show that different organisms and metals have different patterns in metal accumulation and toxicity, which depend on various factors such as species and physiological and environmental conditions. Therefore, data gained from laboratory experiments for each species are important in understanding the relationship between toxicity effects of metal concentrations towards organisms before the organisms can be selected as a bioindicator or as subject in toxicity testing. Further studies of using different species from different taxa also recommended for better understanding protection of Malaysian freshwater aquatic system.

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