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Toxicity of Copper and Cadmium to Freshwater Fishes

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Abstract**—**Two freshwater fishes, *Rasbora sumatrana* (Cyprinidae) and *Poecilia reticulata* (guppy) (Poeciliidae) were exposed for a four-day period in the laboratory condition to a range of copper (Cu) and cadmium (Cd) concentrations. Mortality was assessed and median lethal concentrations (LC_{50}) were calculated. LC_{50} increased with decrease in mean exposure times for both metals. For *R. sumatrana*, LC_{50}s for 24, 48, 72 and 96 hours for Cu were 54.2, 30.3, 18.9 and 5.6 µg/L and for Cd 1440.2, 459.3, 392.3 and 101.6 µg/L respectively. For *P. reticulata*, LC_{50}s for 24, 48, 72 and 96 hours for Cu were 348.9, 145.4, 61.3 and 37.9 µg/L and for Cd 8205.6, 2827.1, 405.8 and 168.1 µg/L, respectively. Results indicated that the Cu was more toxic than Cd to both fishes (Cu>Cd) and *R. sumatrana* was more sensitive than *P. reticulata* to the metals.

Keywords**—**Acute, heavy metals, LT_{50}, toxicity

I. INTRODUCTION

TOXICITY testing has been widely used as a tool to identify suitable organisms as a bioindicator and to derive water quality standards for chemicals. Toxicity testing is an essential tool for assessing the effect and fate of toxicants in aquatic ecosystems. Heavy metals research in Malaysia, especially in using organisms as bioindicators, is still scarce. Therefore, it is important to conduct studies with local organisms that can be used to gain data on metal toxicity, to determine the organism's sensitivity and to derive a permissible limit for Malaysian's water that can protect aquatic communities [1], [2]. Heavy metals such as copper and cadmium have gained wide interest in the scientific community in recent years due to its potential human health hazards. Copper in the form of copper sulfate is used as an algaecide and as a therapeutic chemical for various ectoparasitic and bacterial infections [3] and cadmium, contain in various form of organic and inorganic matter in household and industrial effluents are major contributors to pollution [4]. The purpose of this study was to determine the acute toxicity of Cu and Cd to two local freshwater fish's *R. sumatrana* and *P. reticulata* and to compare the sensitivity between them.

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II. MATERIALS AND METHODS

R. sumatrana were purchased from aquarium shops in Bangi, Selangor, Malaysia and *P. reticulata* were sampled from canals in the university. Prior to toxicity testing, the fishes were acclimatized for one week under laboratory conditions (28-30°C with 12h light:12h darkness) in 50-L stocking tanks using dechlorinated tap water (filtered by several layers of sand and activated carbon; T.C. Sediment Filter®) aerated through an air stone. During acclimation, the fishes were fed with commercial fish food Aquadene®. The standard stock solution (100 mg/L) of Cu was prepared from $CuSO_4.5H_2O$ and Cd was prepared from $CdCl_2.2½H_2O$. The stock solutions were prepared with deionized water in 1-L volumetric flasks. Acute Cu and Cd toxicity experiments were performed for a four-day period using adult animals (approximately 4.0-5.0 and 2.0-3.5 cm body length for *R. sumatrana* and *P. reticulata*, respectively) obtained from stocking tanks. Following a range finding test, five Cu (5.6, 10, 32, 56 and 87 µg/L) and Cd (10, 100, 100, 3200 and 5600 µg/L) concentrations were chosen for *R. sumatrana* and five Cu (10, 100, 320, 560 and 250 µg/L) and Cd (100, 560, 1000, 3200 and 5600 µg/L) concentrations were chosen for *P. reticulata*. Metal solutions were prepared by dilution of a stock solution with dechlorinated tap water. A control with dechlorinated tap water only was also used. The tests were carried out under static conditions with renewal of the solution every two days. Control and metal-treated groups each consisted of five replicates of two randomly allocated fishes in a 500-mL glass beaker (Schott Duran®) containing 400 mL of the appropriate solution. No stress was observed for the fishes in the solution, indicated by 95% survival for the fishes in the control water until the end of the study. A total of 10 animals per treatment were used in the experiment and a total of 110 animals were employed for each species in the investigation. Samples of water for metal analysis taken before and immediately after each solution renewal were acidified to 1% with ARISTAR® nitric acid (65%) before metal analysis by flame or furnace Atomic Absorption Spectrophotometer (AAS – Perkin Elmer model AAnalyst800) depending on the concentrations. To avoid possible contamination, all glassware and equipment used were acid-washed (20% HNO_3), and the accuracy of the analysis was checked against blanks. Procedural blanks and quality control samples made from standard solutions for Cu and Cd were analyzed in every ten samples in order to check for sample accuracy. Percentage recoveries for metals analyses were between 85-105%.

During the toxicity test, the fishes were not fed. The experiments were performed at room temperature of 28-30°C with photoperiod 12h light:12h darkness, using fluorescent lights (334-376 lux). Water quality parameters (pH,

conductivity, and dissolved oxygen) were measured every two days using portable meters (model Hydrolab Quanta®) and water hardness samples (0.45-μm filtered) were fixed with ARISTAR® nitric acid and measured by flame atomic absorption spectrophotometer (AAS– Perkin Elmer model AAnalyst 800). Mortality was recorded every 3 to 4 hours for the first two days and then at 12 to 24 hour intervals throughout the rest of the test period. The criteria used to determine mortality were failure to respond to gentle physical stimulation. Any dead animals were removed immediately.

Median lethal times (LT_{50}) and median lethal concentrations (LC_{50}) for the prawns exposed to Cu and Cd were calculated using measured metal concentrations. FORTRAN programs based on the methods of Litchfield [5] and Litchfield and Wilcoxon [6] 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.

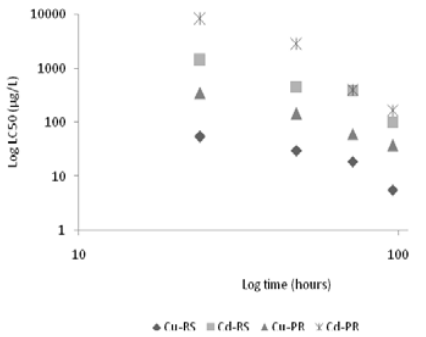

Fig. 1 The relationship between median lethal concentration (LC_{50}) and exposure times for *R. sumatrana* (RS) and *P. reticulata* (PR).

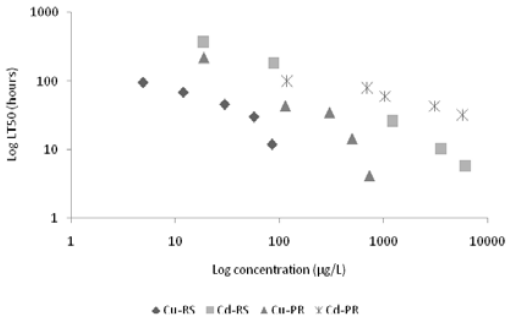

Fig. 2 The relationship between median lethal time (LT_{50}) and metal concentrations for *R. sumatrana* (RS) and *P. reticulata* (PR).

III. RESULTS AND DISCUSSION

In all data analyses, the actual, rather than nominal, Cu and Cd concentrations were used (Table 1). The mean water quality parameters measured during the test were pH 6.50 ± 0.01, conductivity 245.1 ± 0.6 μS/cm, dissolved oxygen 6.30 ± 0.06 mg/L and total hardness (Mg^{2+} and Ca^{2+}) 15.60 ± 2.70 mg/L as $CaCO_3$.

Ninety-five percent of control animals maintained in dechlorinated water survived throughout the experiment. The median lethal times (LT_{50}) and concentrations (LC_{50}) increased with a decrease in mean exposure concentrations and times, respectively, for both metals (Tables 1 and 2). However, the lethal threshold concentration/time could not be determined since the toxicity curves (Figs. 1 and 2) did not become asymptotic to the time or concentration axis within the test period. Figs. 1 and 2 also show that Cu was more toxic than Cd to both fishes.

This study showed that for *R. sumatrana*, LC_{50}s for 24, 48, 72 and 96 hours for Cu were 54.2, 30.3, 18.9 and 5.6 μg/L and for Cd 1440.2, 459.3, 392.3 and 101.6 μg/L respectively. For *P. reticulata*, LC_{50}s for 24, 48, 72 and 96 hours for Cu were 348.9, 145.4, 61.3 and 37.9 μg/L and for Cd 8205.6, 2827.1, 405.8 and 168.1 μg/L, respectively (Table 2). With *P. reticulata*, Park and Heo [7] reported that 24h-LC_{50} for Cu was 1.17 mg/L and Yilmaz et al. [8] reported that 96h-LC_{50} for Cd was 30 mg/L, which were higher than present study. Gomes et al. [9] reported that with juvenil Brazilian indigenous fishes, curimata *Prochilodus vimboides* and piaucu *Leporinus macrocephalus*, 96h-LC_{50} of copper were 0.047 and 0.090 mg/L, and of cadmium 3.16 and 7.42 mg/L for curimatã and piauçu, respectively. The toxicity reported by other studies differs from this study probably due to different species used, aged, size of the organism, test methods and water quality such as water hardness, as this can affect toxicity [10], [11]. In the present study, water hardness was considered low, and the water was categorized as soft water (<75 mg L^{-1} as CaCO3).

A comparison of LC_{50} values indicated that copper was more toxic than cadmium to both fishes and *R. sumatrana* was more sensitive than *P. reticulata* to both metals. Similar results were reported by Gomes et al. [9] with two juvenil Brazilian indigenous fishes which showed that both species were more sensitive to copper than cadmium and Jindal and Verma [12] found that with *Daphnia pulex* the order of toxicity of different metals was Cu>Cd>Ni. However, with other species, cadmium was reported to be more toxic than copper such as to freshwater amphipod *Hyalella azteca* [13], [14], juvenile clams *Mercenaria mercenaria* [15] and juvenile crayfish *Cherax destructor* [16]. This indicates that different organisms have different sensitivity to heavy metals. Possible explanation for this differences to trace metals might be explained as a function of metallothionein (MT) synthesis which believed to provide a protective role against toxic effect of metals in aquatic animals including fishes [17], [18].

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TABLE I
MEDIAN LETHAL TIMES (LT_{50}) FOR *R. sumatrana* AND *P. reticulata* EXPOSED TO DIFFERENT CONCENTRATIONS OF Cu AND Cd.

R. sumatrana

Measured Cu concentration (μg/L)	LT_{50} (h)	95% Confidence limits	Measured Cd concentration (μg/L)	LT_{50} (h)	95% Confidence limits
4.95 ± 2.27	96.15	52.2-177.3	18.5 ± 0.5	371.09	38.5-3581.6
12.06 ± 3.87	68.69	40.9-115.3	88.5± 0.5	183.79	44.7-755.0
30.12 ± 10.65	45.77	27.9-74.9	1223.0 ± 3.8	26.52	11.3-62.4
57.68 ± 14.97	29.91	18.3-48.9	3578.6 ± 13.2	10.46	4.0-27.2
86.02 ± 17.62	11.84	7.7-18.3	6128.4 ± 45.6	5.82	2.6-12.9

P. reticulata

Measured Cu concentration (μg/L)	LT_{50} (h)	95% Confidence limits	Measured Cd concentration (μg/L)	LT_{50} (h)	95% Confidence limits
18.9 ± 0.03	219.49	34.0-1416.1	117.4 ± 0.5	100.74	47.7-212.8
114.0 ± 0.5	43.35	22.5-83.6	691.7 ± 2.3	79.87	39.4-162.0
302.4 ± 1.4	34.69	19.1-62.9	1028.0 ± 14.2	59.49	2.9-59.5
497.7 ± 5.4	14.42	8.0-25.8	3070.9 ± 18.5	42.84	20.3-90.5
728.8 ± 8.7	4.13	2.3-7.1	5701.8 ± 23.3	31.95	16.1-63.3

TABLE II
MEDIAN LETHAL CONCENTRATIONS (LC_{50}) FOR *R. sumatrana* AND *P. reticulata* AT DIFFERENT EXPOSURE TIMES FOR Cu AND Cd.

Time (hour)	LC_{50} (μg/L) for Cu – *R. sumatrna*	95% Confidence limits	LC_{50} (μg/L) for Cd – *R. sumatrana*	95% Confidence limits	LC_{50} (μg/L) for Cu – *P. reticulata*	95% Confidence limits	LC_{50} (μg/L) for Cd – *P. reticulata*	95% Confidence limits
24	54.2	36.2-98.5	1440.2	554.5-3132.3	348.9	243.3-471.0	8205.6	3222.3-9606.4
48	30.3	7.9-48.1	459.3	54.2-1038.6	145.4	72.2-219.36	2827.1	717.3-4564.3
72	18.9	1.7-34.2	392.3	40.3-1021.1	61.3	30.2-102.5	405.8	122.6-1644.1
96	5.6	0.3-10.2	101.6	35.8-288.7	37.9	18.6-68.7	168.1	52.3-610.4