Short Time Effect of Cadmium on Juveniles and Adults of Java Medaka *(Oryzias javanicus)* Fish as a Bioindicator for Ecotoxicological Studies

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Abstract: Acute toxicity of cadmium (Cd) on Java *medaka (Orizyas javanicus)* fish was studied. To obtain the results, the experiments were carried out in 3-liter aquariums (15-20 fish for each aquarium) with static condition based on O.E.C.D method with 5 treatments, and 1 blank in two repetitions. During the experiment, water physico-chemical parameters were pH = 7.7 to 7.9, salinity = 19.3 to 19.7 ppt, temperature = 29.1 to 30.8˚C and conductivity = 13.54 to 13.94 mS/m. On the basis of obtained results, the LC50-96 h for Cd were determined 6.02 (5.83 to 6.21) mg/L for juveniles and 6.63 (6.31 to 6.95) mg/L for adults, respectively. Also, the MAC (maximum allowable concentration) values for Cd on *Orizyas javanicus* juveniles and adults were determined at 0.60 mg/l for juveniles and 0.63 mg/l for adults. These results will be helpful for future ecotoxicology studies based on heavy metals pollution in estuary areas.
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1. Introduction

Increasing of industrial development, uncontrolled population raise, and agricultural water wastes with chemical compounds, particularly heavy elements, cause a lot of industrial and urban sewage to enter the water ecosystem (Duarte *et al*., 2008; Zulkifli *et al*., 2010a; Sow *et al*., 2012a). Thus, heavy metals are considered important and dangerous because they are not analyzed or eliminated from the ecosystem (Pavelieva *et al*., 1990; Zulkifli *et al.*, 2010b). Aquatic animals neutrality have contact with lot of heavy metals and these metallic compounds and concentrations can bring about chemical changes in the earth's crust and human deal's with nature also (Rand, 1995).

The main goal of pollution monitoring is the continual collection of reliable information about the aquatic environment, on those who have a relationship between humans and ecosystem health, as well as control of environmental viewpoint events (Ismail, 1993). In Malaysia, several aquatic fauna have been proposed as bioindicators such as *Perna viridis* (Ismail, 1990), *Oryzias javanicus* (Ismail and Yusof, 2011), *Telescopium telescopium* (Ismail and Safahieh, 2005), *Nerita lineata* (Ismail and Ramli, 1997), *Uca annulipes* (Ismail *et al*., 1991), *Thais* spp. (Mohamat-Yusuff *et al.*, 2010, 2011), *Periophthalmodon schlosseri* (Zulkifli *et al*. 2012) and *Anguilla bicolor bicolor* (Chino *et al*., 2012). Fish have been widely used as a biological bioindicator for pollution monitoring in aquatic ecosystems (Alam *et al.,* 1995; Ismail and Yusof, 2011; Zulkifli *et al*., 2012). Fish can obtain their trace elements, either directly from the water through the gills or indirectly from food through the alimentary tract (Duarte *et al*., 2008; Sow *et al*., 2012b). The mechanisms of transport of metals across the membrane barriers have yet to be fully examined. Cadmium is considered only for its negative effects. The availability of these elements can be influenced by many environmental factors and by composition of diet; as a consequence, the physiological requirements of the essential elements are difficult to determine. In this study we tried to investigate LC50 level of Cd on the Java Medaka fish as a new research organism in the field of ecotoxicology studies in the estuary and coastal areas.

2. Material and Methods

This study was done at the ecotoxicology lab of University Putra Malaysia. After installation of aquariums for
adaptation with new environment, fish that were captured from the Linggi Estuary of Peninsular Malaysia were kept for one week inside aquariums containing 3 liters of natural filtered water from the main Linggi River. In the first step the weight and height of the fish were measured in both adults and juvenile groups.

Experiments were performed according to the standard method of O.E.C.D (organization of Europe Economic Cooperation and Development) (OECD, 1992 &1997) and (TRC and OECD, 1984). Acute toxicity of Cd was done according the statistical way

on the fishes over four days (96hours). Amount of motility was recorded every 24 hours over 96 hours. The fish were divided into groups of 15-20 fish (depending on fish age) and placed in aquariums, each containing three liters Linggi Estuary water and exposed to different concentrations of metal. Mean of weights and heights of fish and concentrations of metal are shown in Table 2.

Every experiment for every age was done twice and Cd was ordered in five concentrations. There was one aquarium without any concentration of any metal as a control group. Every 24 hours, the aquariums were evaluated and the mortality recorded every day at the same time over 96 hours. (Tables 3a & 3b).

In the experiment period the fish were not fed and physic-chemical parameters such as temperature, pH and dissolved O_2 were

measured also (Table 1). Behaviors and positions of the fish were recorded and dead fish were immediately removed from the aquarium. After 4 days and on completion of the first experiment, water of the aquariums was emptied and the aquariums washed using salty water and double dissolved water prepared for the second experiment. After repeating the experiment various results were recorded, including the amount of LC_{50} Cd in 48 and 96 hours, determined by using Probit statistical analysis method (Finney, 1990), with attention on the metals concentrations used and recorded for motility amounts (Table 4).

3. Results

According to the said method, the acute toxicity test (LC50) was done followed by the recording of fish mortality in metal concentration at different times (every 24 hours). Meanwhile, for greater focus and less error, the top test was done in two repeats and the results were found to be almost similar in both experiments (Table 2). In the end, results were analyzed using SPSS package and Probit Analysis method (Table 4).

Fish behaviors at different times of the experiment were observed and recorded: when they were excited and showed unusual stir, unnatural discharge on the skin and body, and slow movement to relatively complete stop (dying in the continuous position). Maximum Allowable Toxicant Concentration (MATC) was determined with attention to LC50 amounts also (TRC and OECD, 1984). Maximum allowable toxicant concentration is the concentration that has no negative effect on fish in the experiment period. Amounts of NOEC and LOEC were determined in this study with attention to LC50 amounts also. NOEC or No Observed Effect Concentration in a toxicity experiment is the maximum concentration of a substance that has no statistical effect on the population of samples when compared with the control group. LOEC or Lowest Observed Effect Concentration in a toxicity experiment is the least concentration of substance that has no statistical effect on the population of samples when compared with the control group. The maximum allowable toxicity concentration is higher than the lowest observed effect concentration and is lower than no observed effect concentration also (NOEC <MATC <LOEC).

According to the results of the acute toxicity test of cadmium on the adults and juveniles of Java Medaka fish, the amount of lethal concentration of Cd in the 4 days (96 hours) for 50% of the fish were 6.63 for adults and 0.60 for juveniles respectively. In comparing results between adults and juveniles it was observed that there was no high difference of Cd amounts between 96 hours results in adults and juveniles, meaning this metal (Cd) is a toxic and killer for both adult and juveniles in low concentration and at same levels. Fish exposed to Cd did not show any unnatural signals but after a while they showed a state of restlessness, unbalanced and fast swimming. Then they skin color took on certain brightness, with mucus on the skin increased while curvature of the spine and gill hyperemia was noticed in some of fish.

Results of researches about acute toxicity test effects on fish indicate that some fresh water and marine fish are comparable to Java Medaka in terms of acute toxicity test results. Researches about Cd show that LC50-96h this metal for Common Carp is 2.84 and 4.55 mg/L respectively for juveniles and adults (Ramesha *et al*., 1996), for Trout fish and Salmon were 0.32 and 3.11 mg/L respectively (Mance, 1987) and for finger height fish of Phytofag and Amor respectively were 0.721 and 1.04 mg/L. Also LC50 of Cd for juveniles of Rohu (*Labeo rohita*) were 89.5 mg/L (Dutta *et al*., 2001) and 2.5 mg/L for Flag fish (Sphar., 1996). In comparing LC50 of Cd in the above species with Java Medaka fish, the position of Java Medaka fish is:

Rohu < Java Medaka (Adult) < Java Medaka (Juvenile) < Common Carp < Salmon < Flagfish < Amor < Phytofag < Trut fish

Small fish like Killifish and Medaka fish have been suitable models for ecotoxicology studies in different researches (Inoue and Takei, 2002). In another study, it was reported that LC50-96h, 4-chlorophenol, zinc, hexavalent chromium and residue chlorine were from 3.0 to 4.1, from 12.4 to 14.7, 7.4 and 0.05 mg/L respectively (Koyama *et al*., 2008).

Table1 – Data range of physico-chemical parameters in treatment aquaria

Parameter	Salinity (ppt)	pH	Temperature (°C)	Conductivity (mS/m)	O_2(mg/L)
Mean	19.3 – 19.7	7.7– 7.9	29.1 – 30.8	13.54-13.94	6.7-6.9

Table2: Mean of weight and height and Cd concentrations in adults and juveniles of *O. javanicus*

	Juveniles	Adults
Number of fish	15	10
Mean of length	1.4±0.3 cm	2.8±0.3 cm
Mean of weight	0.099±0.03 g	0.213±0.05 g
Range of heavy metal concentration	1-15 mg/L	1-15 mg/L

Table 3a: Number of dead *O. javanicus* adults in the 4 days for Cd in two repeats

Cd (I) / time	Control	1(mg/L)	3.5(mg/L)	7(mg/L)	11(mg/L)	15(mg/L)
24	0	0	0	0	0	1
48	0	0	2	1	2	2
72	0	0	0	0	8	7
96	0	3	2	4	-	-
Cd (II) / time	**Control**	**1(mg/L)**	**3.5(mg/L)**	**7(mg/L)**	**11(mg/L)**	**15(mg/L)**
24	0	0	0	0	0	1
48	0	0	1	1	3	4
72	1	2	2	1	7	5
96	0	1	2	3	1	-

Table 3b: Number of dead *O. javanicus* juveniles in the 4 days for Cd in two repeats.

Cd (I) / time	Control	1(mg/L)	3.5(mg/L)	7(mg/L)	11(mg/L)	15(mg/L)
24	0	0	0	0	4	3
48	0	0	1	2	4	6
72	0	2	3	2	7	6
96	1	3	2	4	-	-
Cd II) / time	**Control**	**1(mg/L)**	**3.5(mg/L)**	**7(mg/L)**	**11(mg/L)**	**15(mg/L)**
24	0	0	0	0	3	4
48	0	1	2	3	5	7
72	0	2	3	2	7	4
96	0	2	3	3	-	-

Table 4:LC50 Results of Cd for adults and juveniles of *O. javanicus* in two repeats

Subject	Probit Line	Estimate of log (LC50)	Estimate of LC50 (mg/L)	SE of log (LC50)	95% c.l. for Log (LC50)	95% c.l. for LC50
Cd.48h Adult-1	Y = 2.0011 X + 2.8161	1.09	13.34	0.13	0.88, 1.85	7.61, 71.77
Cd.48h Adult-2	Y = 1.2923 X + 3.5146	1.15	13.90	0.21	0.84, 2.49	7.00, 68.7
Cd.96h Adult-1	Y= 1.3463X + 3.8659	0.84	6.96	0.15	0.52, 1.35	3.11, 16.6
Cd.96h Adult-2	Y= 1.4322 + 3.8537	0.80	6.32	0.14	0.49, 1.22	3.11, 16.6
Cd.48h Juvenile-1	Y = 2.6305 X + 2.1629	1.08	11.98	0.10	0.91 , 1.51	8.21, 32.86
Cd.48h Juvenile-2	Y = 1.2923 X + 3.5146	1.15	13.11	0.21	0.85, 2.50	7.02, 53.7
Cd.96h Juvenile-1	Y = 1.5307 X + 3.8276	0.77	5.83	0.13	0.47, 1.14	2.96, 13.7
Cd.96h Juvenile-2	Y = 1.4888 X + 3.8182	0.79	6.22	0.14	0.50, 1.22	3.14, 16.5

Java Medaka did not show higher sensitivity for chemicals than fresh water fish (Koyama *et al*., 2008). It is also reported that LC50-48h on one type of killifish named *Fundulus heteroclitus* for Zn, Cu and Cd were determined at 96.5, 44.4 and 19.0 mg/L respectively (Burton *et al*., 1990).

One of the most important events of environmental pollution depends on heavy metals and industrial discharges, which are important contributing source of pollutants in the ecosystems (Imai *et al*., 2005). The use of bioindicator is proven to be very useful. To obtain quick information on ecotoxicology, short-term tests are more useful than long-term tests. These tests are used to determine total toxicity of solutions when there is no information about their toxicity levels. Moreover to assess different organisms' answers to toxicity tests, knowing the different positions of some parameters such as temperature and pH is necessary.

4. Conclusion

An amount of LC50 is a useful criterion for acute toxicity but it is not a representative amount of pollutants substances in aquatic habitats. Content of waste materials that are not toxic in 96 hours, will be harmful in the longer run inside a water habitat, thus LC96h may be seen only in some parts of long-term toxicity. According to these study results, the indications are that Java Medaka fish is a suitable species for ecotoxicological study and it is introduced as an important fish in the toxicity tests because it is easy to grow under laboratory conditions. Additionally, this fish can be useful for testing the endocrine effect of chemicals in long-term as well as all period of life.

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