Heavy metal cadmium (Cd) content in the medaka fish
*Oryzeas javanicus* Bleeker

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Abstract. The medaka oryzeas fish *Oryzeas javanicus* Bleeker has been widely used as bioindicator animal. The present study aims to assess a lethal dose 50% (LD50) of heavy metal Cadmiun (Cd) towards test animal oryzeas fish. Fish was collected from the estuary of Jeneberang river of Makassar. Samples were acclimatized in a laboratory before being a test. Randomized Complete Design with four treatments and four replicates was applied in this study. Test fish was treated without (control) and with three different levels of Cd concentration as follows 0 mg / L; 0.008 mg / L; 0.01 mg / L and 0.03 mg/L. An observation was done during seven days experiment. Observed parameters included a change in movement, morphological abnormalities (peeled skin) as Cd content in medaka fish. Least Significance Different (LSD) method was applied to see different significant effect amongst treatments. At the end of the experiment, Cd content in test fish was determined by using an Atomic Absorption Spectrophotometer (AAS). The result indicates that medaka fish Oryzeas javanicus showed disorientation movement since day 5 at Cd concentration 0.03 mg / L and increased to the end of the test. Peeled skin happen at day seven at all three treatments (Cd 0.008; 0.01 dan 0.03 mg/L). Cd content in fish was low at concentration of 0.008 mg/L (average 1.50 mg/kg) and the highest at Cd 0.03 mg/L (average 4.19 mg/kg), respectively. Statistical analysis reveals that Cd concentration 0.03 mg/L differs from other treatments. Level of Cd accumulation in *O. Javanicus* Bleeker increased with an increase in metal concentration in water.

1. Introduction

Water pollution is the inclusion of living creatures, substances, energy or other components into water bodies causing water quality polluted. Pollutants may include heavy metal. Metal can accumulate in aquatic organisms, including fish and persist in water and sediment. Nowadays, contamination by heavy metals in waters such as cadmium (Cd), mercury (Hg) and lead (Pb) become a serious threat. Generally, heavy metal at room temperature is not always solid, but in the form of a liquid element, for example, mercury (Hg), lead (Pb), and Cadmium (Cd). Mercury, cadmium, copper, lead and zinc are of the most important pollutants in the aquatic environment and fish [1]. Cadmium is recognized as white silvery soft metal that is easily oxidized [2]. This metal can be found from zinc mining waste, electroplating flush, waste sediment, and metal industries. Widely used of this metal can also lead to Cd pollution into the environment.

Measurement of the levels of contamination, or the detection of pollutants in water, generally using chemical techniques. These techniques tend to be expensive and wastes can pose new pollutants to the environment. Currently, developing techniques for early detection of pollution substances or chemicals in the water using biological indicators. In this study we want to know the type of biological
indicators for water pollution decent and easy to obtain. One that meets the requirements of aquatic organisms as biological indicators are medaka fish. Oryzeas fish can be one of the biological indicators that because they can live in the pressure of heavy metals in waters, have the high reproductive ability, spawning time is relatively short, the level of adaptation high that can live in freshwater and seawater, settled on a body of water so easily made sample, can be easy developed in the laboratory. The biologic impact of toxic pollutants on fish is an important area of study in ecotoxicology. Fish models, such as zebrafish (*Danio rerio*), tilapia (*Oreochromis niloticus*), and rainbow trout (*Oncorhynchus mykiss*), medaka fish, have been widely used for ecotoxicological studies in the freshwater and marine environment [3][4][5]. Zebra fish and medaka fish has been used as a model organism for modern biomedical research [6] and also transgenic zebra fish can be served as bioindicators of environmental toxicants at the cellular level [7]. Fish used in this study is medaka javanicus *Oryzias javanicus* as a local endemic fish which are found in South Sulawesi and is also easily to be cultivated. Therefore, research on the use of this fish as biological indicators in polluted waters is needed.

2. Material and Method

2.1. Materials
Equipment used in laboratory included glass jar measuring 10 x 10 x 10 cm, aerators, measuring devices, digital cameras, sample boxes, petridish, scales, horn spoons, scissors, gill net and AAS. Materials used in this study included medaka javanicus *Oryzias javanicus* fish, Cadmium (Cd) solution with a concentration of 0.01, 0.03, and 0.05 mg/L, glass glue, water, pellets, board marker, and rolls tissue.

2.2. Sample collection.
This study was conducted in May-August 2016. Sampling was conducted at the mouth of the river otters Je'ne Berang, Makassar of South Sulawesi province. Acclimatization and experiment were done at Medaka Study Center, the 5th Floor Research Center Building Hasanuddin University, while Cd content was analyzed Department Chemistry Faculty of Mathematics and Natural Sciences Hasanuddin University Makassar. Prior to sampling, an observation was done in order to get a general idea of the condition of estuary waters Je'ne Berang, Makassar, South Sulawesi. Sampling was done randomly using a gill net. Collected fish was photographed for documentation and samples were taken to the laboratory. Fish was sorted accordingly, with a size of 2 ± 0.1 / 0.2 cm.

2.3. Acclimatization
Fourthly animal test that had been sorted, adapted to the physical conditions in the laboratory (testing environment) for 10 days. Acclimation for at least 10 days because if within 48 hours more than 3% of the population of test animals die, the population of test animals deemed ineligible for testing.

2.4. Experimental design
LD$_{50}$ test was conducted prior to application of different concentration of Cd solution as the main task of this study. The lethal doses of Cd with varying concentrations of 0.75, 1, 1.25 and 1.5 mg/L were administered towards test fishes. Mortality (%) of test animals was recorded after 24. Experiment on heavy metals Cd content was done using a completely randomized design (RAL), which consists of 4 treatments and 4 repetitions as follows:
- Treatment (tr) 1: No additional Cd (0 mg / L) (control).
- Treatment (tr) 2: 0.008 mg / L Cd (below the threshold).
- Treatment (tr) 3: 0.01 mg / L Cd (threshold).
- Treatment (tr) 4: 0.03 mg / L Cd (above the threshold).

Cd content in test animals was measured using Atomic Absorbance Spectrophotometer (AAS) at the wave length of 248.3 nm. At the end of the experiment, test animal was crushed, homogeneous and analyses according to the AAS method elsewhere.
2.5. Data Analysis
Data analysis consists of Cd content in the test fish as abnormalities in fish movement. Parameters in fish movement such as disorientation characterized by weakened fish movement and fish body becomes tilted position, as well as abnormalities characterized by morphological changes in fish especially change in gills colour into red and flaky fish scales.

3. Result and Discussion

3.1 Lethal Dose 50% (LD$_{50}$)
The result of the LD$_{50}$ test for oryzeas fish is shown in Figure 1. As seen in the Figure, at the concentration of 1.25 mg/L Cd, 50% of the fish population died after 24h. This reveals that LD$_{50}$ for Oryzias javanicus Bleeker, respectively.

![Figure 1. LD$_{50}$ after 24 h](image)

LD$_{50}$ is an initial test to observe acute toxicity potential, toxicity symptom, toxicity spectrum and dying mechanism from a toxic agent. LD$_{50}$ aims to detect toxicity of material, target organ and its sensitivity, to obtain data on the effect on the acute chemical, as to initial information on target dose [8].

3.2 Effect on Cadmium on Oryzeas javanicus Bleeker
The effect on the different concentration of Cadmium in the water on fish O. Javanicus Bleeker abnormalities (movement and skin) is described in Table 1.

| Time (h) | Control (n = 12) | Cd 0.008 mg/L (n = 12) | Cd 0.01 mg/L (n = 12) | Cd 0.03 mg/L (n = 12) |
|---------|-----------------|------------------------|-----------------------|------------------------|
|         | DM  | LS  | DM  | LS  | DM  | LS  | DM  | LS  |
| 0       | -   | -   | -   | -   | -   | -   | -   | -   |
| 24      | -   | -   | -   | -   | -   | -   | -   | -   |
| 48      | -   | -   | -   | -   | -   | -   | -   | -   |
| 72      | -   | -   | -   | -   | -   | -   | -   | -   |
| 96      | -   | -   | -   | -   | -   | -   | -   | -   |
| 120     | -   | -   | -   | -   | -   | +   | -   | -   |
| 144     | -   | -   | +   | -   | + (2) | - | + (2) | - |
| 165     | -   | -   | + (2) | + | + (3) | + (2) | + (5) | + (3) |

Table 1. The relationship between time and number of fish that show abnormalities [disoriented movement (DM) and peeled skin (PS)].
Percent (%)  0  0  17  8  25  16  41  25

Note:
\( n \) = number of fish
(-) = no abnormalities
(+) = number of abnormal fish

As shown in Table 1, no abnormalities are shown by test animal at all concentration before 120h after the experiment started. One fish starts to show disoriented move at day 5 (120h) at the highest Cd concentration. At the highest concentration, the number of fish show disorientation movements increase towards the end of the experiment. Overall, percentage of disorientated fish increases with an increase in Cd concentration, that of Cd 0.008 mg/L (17%); Cd 0.01 mg/L (25%) and Cd 0.03 mg/L (41%). As also shown in Table 1, scale abnormality (Figure 2) happened at last day of the experiment at the lowest Cd concentration (0.008 mg/L), and a number of fish that shows similar abnormality increases as an increase in Cd concentration.

![Figure 2. Scale abnormality (peeled skin) on the test animal](image)

Heavy metal enters organisms through food, air respiration, as also through the skin [2]. A present of heavy metal in water or sediment, hence the ability of organisms to accumulate it inside their body, will affect existing of organisms [9].

Pathophysiological effect on fish can be shown by a change in fish behaviours such as movement, body balance, mucus production and color change. The present of Cd in fish media in a small amount can affect biological activity that can lead to the dead of organisms [10]. Accumulation of pollutant for example any heavy metal in fish is allowed when one is exposed to such metal. However, the effect of any heavy metal pollutant to organisms is depended on the toxicity of that heavy metal and a successful detoxification and excretion. An increased level of heavy metal inside fish would disturb organs function, and when it over a threshold, the fish organ will be damaged.

Study done by [11], on the accumulation of Cd content in fresh water shell, concluded that, biotransformation and bioaccumulation happened inside water’s organisms. Cd enters organisms through food or water, being accumulated in gills, digestive tract, even in a kidney. A bio-magnification process can also allow an amount of such metal is higher inside organ compared to the surrounding water. Carcinogen Cd gives a slowly toxic effect, when heavy metal content over the threshold level, this can lead to a dead. Toxic effect is depended on the level of contaminant, for example, moderate level results in physic weakness, loose appetite, un-normal breath, and un-control swimming behavior. Fish is categories as bio-indicator of chemical pollutant as in the certain level of pollutant concentrations, as can show a change in movement, unusual growth or dead.

3.3 Cd content in test fish
The result of Cd content analysis in test fish is given in Table 2, while the result of statistical analysis amongst treatments is shown in Table 3. As shown in Table 2, Cadmium content in test fish increase with an increase of Cd concentration in media. Average of Cd content in different Cd concentration as follow 0.50 mg/kg (tr 1); 0.61 mg/kg (tr 2) and 1.39 mg/kg (tr 3) (Table 2). Table 3 indicates that there is no significant different between control with tr 1 (Cd 0.008 mg/L) and tr 2 (Cd 0.01 mg/L) but differs to treatment 3 (Cd 0.03 mg/L).

| Table 2. Cadmium content in *Oryzias javanicus* Bleeker |
|----------------------------------------------------------|
| Treatment (tr)     | Cd content (mg/kg) | Average Cd content (mg/kg)/fish |
|Cd (mg/L)           | I    | II   | III  | IV   |                      |
|Control             | 0.22 | 0.67 | 0.00 | 0.00 | 0.07                 |
|0.008               | 0.81 | 1.32 | 3.05 | 0.83 | 0.50                 |
|0.01                | 0.92 | 1.49 | 3.28 | 1.63 | 0.61                 |
|0.03                | 7.06 | 2.23 | 3.99 | 3.49 | 1.39                 |

| Table 3. The result of Least Significance Different (LSD) analysis for Cd accumulation in *Oryzias javanicus* Bleeker |
|------------------------------------------------------------------------------------------------------------------|
| Treatment (tr) | Average | LSD value at 5 % =1.93 |
|Cd 0.008 mg/L   | 1.50ns  |                        |
|Cd 0.01 mg/L    | 1.83ns  |                        |
|Cd 0.03 mg/L    | 4.19*   |                        |

Note: ns = not significant * = Significant

Heavy metal can be accumulated inside fish, in which this will form a complex compound with organic material from fish itself. Hence, heavy metal will be fixed and cannot be excreted and being accumulated inside a fish body [12]. Heavy metal accumulated inside a body, to some extent higher compared its level in the environment. As shown in ANOVA analysis and LSD, a higher level of Cd content inside media, the higher amount is being accumulated inside fish. The relationship between metal absorption in an organism is in accordance with its level in media, in which an increase of metal content in tissues follows an increased level in the environment.

Naturally, heavy metals are present in freshwater, brackish water, and marine environment. However, human activities have also contributed to heavy metal pollution in a different ecosystem. Heavy metals pollutant comes from industrial wastes as domestic wastes [13]. This study has shown that oryzeas fish can be used as bio-indicator of heavy metal Cd in an environment.

4. Conclusion

1. Medaka Javanicus *O. Javanicus* Bleeker show sub-lethal sensitivity towards heavy metal Cadmium (Cd) in form of disoriented move at day-5 and increase with an increase of exposure time as scale abnormality at day-7.

2. Cd accumulation in Medaka fish Javanicus *O. Javanicus* Bleeker in accordance with concentration in media.

References

[1] Authman MMN, Zaki MS, Khallaf EA, Abbas HH 2015 *J Aquac Res Development* 6(4). [http://dx.doi.org/10.4172/2155-9546.1000328](http://dx.doi.org/10.4172/2155-9546.1000328)

[2] Palar H 2008 *Pencemaran dan Toksikologi Logam Berat* Penerbit Rineka Cipta Jakarta.

[3] Dong S, Kang M, Wu X, Ye T 2014 *Development of a promising fish model (Oryzias melastigma) for Assessing Multiple Responses to Stresses in the Marine Environment.*
[4] Ishikawa Y 2000 Medakafish as a Model System For Vertebrate Development Genetics. Bioessays 22(5):487-495

[5] Andriani I, Litaay M, Sartika, Tahir D 2019 Medaka Fish Oryzias javanicus Bleeker as bio-indicator of Lead (Pb) content in waters. Paper presented at The 3rd ICOS 26-27 July 2019. Makassar.

[6] Lin CY, Chiang CY, Tsai HJ 2016 Journal of Biomedical Science 23(19) DOI 10.1186/s12929-016-0236-5.

[7] Tsai HJ 2017 Transgenic ZebraFish can serve as Bioindicator of Enviromental Toxicants at the Cellular Level. Ann Mar Biol Res 4(2): 1022.

[8] Ibrahim M 2012 Dose 50 % (LD50) of poliherbal (Curcuma xanthorriza, Rheinhovia hospita, Nigella sativa, Arcangelisia flava and Ophioccephalus striatus) on heparmin Mus Musculus. Research And Development. PT Royal Medicalink Pharmalab. 4-5.

[9] Jagfar A, Abdul M 2014 Deteksi logam Timbal (Pb) pada ikan Nila sepanjang Sungai Kali Mas. Jurnal Ilmiah Perikanan dan Kelautan 6(1): 42-48.

[10] Dewi NK 2004 Decrease toxicity level of cadmium in Chanos chanos Forskal using Eichornia Crassipes (Mart.) Solms and its transport phenomena. Thesis. Universitas Diponegoro, Semarang.

[11] Sunarto 2007 Bioindicator pollutant cadmium (Cd) using microanatomi structure analysis, gills function efisiensi, morphology and shell condition of fresh water shell Anodonta woodiana Lea). Dissertation. Universitas Airlangga. Surabaya.

[12] Ansari TM, Marr I L, Tarig N 2004 Heavy metals in Marine Pollutan Perspective Mini Review. Journal Applied Science 4(1):1-20.

[13] Casas JS, Sordo J 2006 Lead, Chemistry, Analytical Aspects, Environmental Impac and Health Effects. Departamento de Quimica Inorganica Facultad de Farmacia, Universidad de Santiago Compostela, Galicia, Spain.