Medaka fish *Oryzias javanicus* Bleeker as bio-indicator of lead content in waters

I Andriani¹, M Litaay², Sartika³ and D Tahir⁴

¹,²,³Department of Biology Faculty Mathematics and Natural Science, Hasanuddin University, Indonesia 90245
⁴Department of Physic Faculty Mathematics and Natural Science, Hasanuddin University, Indonesia 90245

Email corresponding: mlitaay@fmipa.unhas.ac.id

**Abstract.** An increase of heavy metal used in various products has lead to an increase of its pollution in water column elsewhere. Previous studies have shown that member of medaka fish is widely used as bio-indicator of pollution. Various medaka fish have been used in environmental as medical studies. The present study aims to observe capability of medaka fish *Oryzias javanicus* Bleeker as bio-indicator of lead [Pb] content in waters. Fish was collected from the mouth river of Jeneberang South Sulawesi and acclimatized at the Medaka Study Centre of Hasanuddin University. In order to study capability as bio-indicator, the fishes as test animals was treated with different concentration that of 0; 0.01; 0.03 and 0.05 mg/L Pb. Test animals were kept in media containing Pb for ten days before assessing their metal content. Pb content in animals was calculated after treatments. Composite method was applied for Pb content in medaka fish, in which fish was crushed and proceed to metal content analysis using EDXRF spectrofotometer. The result indicates that medaka fish *Oryzias javanicus* Bleeker survives at threshold concentration 0.05 mg/L Pb, respectively. We conclude that *Oryzias javanicus* can be used as bio-indicator of lead content in waters.

1. Introduction

Pollution by some heavy metals in waters is a serious threat to the balance of ecosystems and human life. Generally heavy metals at room temperature are not always in solid form but there are in the form of liquid elements. Heavy metals such as mercury [Hg], lead [Pb], kadium [Cd], and others that exposed to the water continuously will accumulate and will pollute the water area. However, so far water pollution had been detected when the impact of pollution had occurred in the form of changes in the physical properties of the waters or the death of organisms living in these waters even after the appearance of death in humans [1]. This phenomenon is certainly dangerous for the survival of human being. Therefore, an early detection effort is needed on the presence of pollutants in water.

Generally, pollutants are detected in the waters using chemical techniques. This technique tends to be expensive and the waste can cause new pollutants for the environment. Currently, people developed early detection techniques for pollution of substances or chemicals in the waters by using biological indicators. In this study, we want to know the types of biological indicators for water pollution that are feasible and easily obtained. One of the aquatic organisms that meet the requirements as a biological indicator is medaka fish. Medaka fish can be one of the biological indicator agents because it can live in the pressure of heavy metals in the water, has high reproductive ability, relatively short spawning
time, few of them have high adaptability that can live in freshwater and sea water, settling in a waters so it is easy to make samples, easy to develop in the laboratory. In this study, we would like to observe the potency of medaka fish species from Indonesia and found cosmopolite in South Sulawesi, as well as easily cultivated, that is *Oryzias javanicus*.

2. Material and Methods
2.1 Materials
The tools used in this study were 10 x 10 x 10 cm glass jars, aerators, measuring instruments, digital cameras, sample boxes, petri dishes, scales, horn spoons, scissors, X-RAY instruments and gill net. The material used in this study was a sample of *O.javanicus* fish with approximately 2 cm standard length, Pb lead solution with concentrations of 0.01, 0.03, and 0.05 mg / L, glass glue, tap water, pellets, boardmarkers, and rolled tissue.

2.2 Acclimatization
There were 40 fish selected to be models. They adapted to the physical conditions in the laboratory for 10 days minimum. Johnson [2] said that within 48 hours more than 3% of the population of the animal dies, then the population of the *O.javanicus* is considered not eligible for testing. Prior to the acclimatization process, Pb heavy metal content testing was carried out on tap water.

2.3 Experimental design
The study began with searching LD 50 test for *O.javanicus* as test animal in different concentrations of 0.5; 0.75; 1 and 1.25 mg/L Pb administered towards fishes.

The present study used a completely randomized design, consisted of 4 treatments [Tr] and 2 repetitions as follows:

- Tr 1. Pb 0 mg/L Pb [control].
- Tr 2. Pb 0.01 mg/L Pb [below the threshold].
- Tr 3. 0.03 mg/L Pb [threshold].
- Tr 4. 0.05 mg/L Pb [above the threshold].

Lead content in *O.javanicus* was measured using EDXRF spectrofotometer instrument. At the end of experiment, test animal were crushed, homogeneous and inserted into the irradiation container to be analyzed by EDXRF spectrofotometer.

2.4 Data analysis
Data analysis included level of Pb content in the fish and abnormalities in fish movement. Parameters in fish movement such as disorientation characterized by weakened fish movement and fish body becomes tilted position, morphological changes in fish especially change in gills color into red and flaky fish scales.

3. Results and Discussion
3.1 Pb content in media used for acclimatization
The result of Pb levels in tap water that used as a media water acclimatization shows that the water contained no heavy metals Lead [Pb] so that the water can be used in this study. The results on the tap water analysis is shown in table 1.

| No | Element | m/m% | StdErr |
|----|---------|------|--------|
| 1  | U       | 46,20| 14,67  |
| 2  | Sr      | 16,34| 7,48   |
| 3  | In      | 13,46| 3,85   |

Known Conc = 0  REST = 0  D/S = 0
Sum Conc’s before normalization to 100% = 0,2 %
As shown in the table 1, the elements of metal detected by means of X-ray that is U, Sr and inclusive with different masses and different standard error. While heavy metals Pb was not detected so that the waters used during acclimatization were free from lead.

### 3.2 Lethal Dose 50% [LD 50]

The LD 50 test showed percentage mortality in *O.javanicus* against different concentration of Pb as shown at figure 1.

![Figure 1](image-url)

**Figure 1.** Percentage mortality in *O.javanicus* after 24 hours [Y-axis] at different concentrations [X-axis].

The result indicated that at the concentration 0.5 mg/L Pb at 24 hours observation, percentage mortality of *O. javanicus* was 0%, mortality at a concentration of 0.75 mg/L was 25%. While Pb concentration of 1 mg / L within 24 hours as seen in the graph, animals survived up to 50%. It can be concluded that the LD 50 lethal dose Pb after 24 hours for *O. javanicus* was 1 mg/L. Acute toxicity tests are designed to determine or indicate roughly the median lethal dose [LD50] of toxicant. LD50 defined as a sign of statistics on put a single dose of a substance that can cause death of 50% of test animals [3]. The author added that number of death was used as a measure of test animals to the toxic effects of a substance [chemical] on a group of test animals. If in this case test animal seen as a subjects, such as death response is a discrete response. It means there are only two kinds of responses that is there or not there is death.

Acute toxicity was defined as the occurrence of poisoning due to exposure to toxic materials in a short time, which is usually calculated by using LC50 or LD50 values. This value is obtained through statistical process and serves to measure the relative number of chemicals acute toxicity. Acute toxicity of environmental chemicals can be determined experimentally using certain species such as mammals, the nation's poultry, fish, invertebrates, vascular plants and algae. Acute toxicity tests may use some mammals, but it is recommended to LD50 test such rats, mice and rabbits. LD50 test is a test to establish the potential for acute toxicity LD50, assessing any toxic symptoms, the spectrum of toxic effects and mechanism of death.

### 3.3 Effect of Pb on *O. javanicus*

The result of the effect of Pb on morphology and movements of *O. javanicus* are shown in table 2. It is obvious from table 2, no disoriented move and abnormalities is shown in organ of *O.javanicus* at control. At treatment 1, disoriented motion and organs abnormalities is noticed after 120 hour after treatment. Fishes treated with threshold concentration of 0.03 mg/L shown abnormalities as early at 96 hour after being treated. This fish showed oblique body position, gills red and flaky scales. Number of fish that show morphological changes and disorientation move increased to 2 tails after 168 hours of observation [day 7] at this treatment. As Pb concentration increases, at the highest Pb content [0.05 mg/L], one fish showed disoriented as abnormalities at 24 h treatment. However, its movement become
normal afterwards. This may due to adaptation to water quality. After 120 hours after being treated until the end of experiment, two fishes showed disoriented movement as abnormalities.

**Table 2.** The effect of Pb at different concentration level on the movement and on gills and scales of *Oryzias javanicus*.

| Time [hour] | Control | 0.01 mg/L | 0.03 mg/L | 0.05 mg/L |
|------------|---------|-----------|-----------|-----------|
|            | DM Abnormalities | DM Abnormalities | DM Abnormalities | DM Abnormalities |
|            | Gills Scales | Gills scales | gills scales | gills scales |
| 0          | - - - - - - | - - - - - - | - - - - - - | - - - - - - |
| 24         | - - - - - - | - - - - - - | - - - - - - | + + + + + + |
| 48         | - - - - - - | - - - - - - | - - - - - - | - - - - - - |
| 72         | - - - - - - | - - - - - - | - - - - - - | - - - - - - |
| 96         | - - - - - - | - - - - - - | - - - - - - | + + + + + + |
| 120        | - - - - - - | - - - - - - | - - - - - - | + [2] + [2] + [2] |
| 144        | - - - - - - | - - - - - - | - - - - - - | + [2] + [2] + [2] |
| 168        | - - - - - - | - - - - - - | - - - - - - | + [2] + [2] + [2] |

Note:

[+] The fish are disoriented motion and abnormalities.
[-] All normal fish [10 fish].
+ [2] two fish show disoriented motion and abnormalities.
DM : disoriented motion

The effect of heavy metal long exposure can changes patho-morphology form. The morphologic changes to fish branchia histological damage can be attributed to the level of pollution of water where the fish live and / or discovered [4]. Disorientation picture and abnormalities are seen in figure 2.

![Figure 2](image.png)

**Figure 2.** The animal test of *Oryzias javanicus* (black arrow) show disoriented motion after treatment 4.

As reported previously by other author that the toxicity of lead [Pb] to aquatic organisms may cause damage to the tissues of organisms, especially on sensitive organs such as gills and intestines and then to the inner tissues such as the liver and kidneys where the metal accumulates [5]. Abortion
also found in the network continues to increase in accordance with increase in the concentration of lead in the water and the length of the organism is in contaminated waters Pb [6]. This is because the aquatic organisms are not able to regulate heavy metals Pb organisms that enter the body. Toxic pollutants can lead to death [lethal] and not death [sub-lethal], for example, disruption of growth, behavioral and morphological characteristics of various aquatic organisms [7]. Lead also can induce oxidative damage through direct effects on the cell membrane, interactions between lead and haemoglobin, which increase the auto-oxidation of haemoglobin, auto-oxidized δ-aminolevulinic acid, interactions with GR, or through the formation of complexes with selenium, which decrease GPx activity [8]. Sevcikova et al. summarized the results of several studies, he argued that the manner and duration of exposure are important factors in lead-induced oxidative stress [9].

3.4 Lead content in *O. javanicus*

Levels of lead content were detected by EDXRF spectrofometer and the results were described in table 3. As seen in table 3, no Pb was detected in control, while average of Pb content in other treatments various form 0.00084 to 00095 mg/L.

| No | Pb [mg/L] | Pb content [mg/L] | Average [mg/L] |
|----|-----------|--------------------|----------------|
|    |           | R I                | R II           |
| 1  | Control   | 0                  | 0              |
| 2  | 0.01      | 0.00084            | 0.00083        | 0.00084 |
| 3  | 0.03      | 0.00093            | 0.00086        | 0.00089 |
| 4  | 0.05      | 0.00094            | 0.00095        | 0.00095 |

Heavy metals in the water can be absorbed and accumulated by body tissues of aquatic biota through water and the food chain. Pollution in water bodies including rivers, will cause problems and have a negative impact on the lives of the fish that live in the river. Polluted waters will experience a loss of quality, which led to decreased water carrying capacity of the aquatic organisms that live in it. Water pollution problems cause various effects, good effects of biological, physical or chemical. Toxic substances can reduce the growth rate [10]. A decrease in the growth rate of the fish suspected impaired organ thereby reducing appetite and utilization of energy derived from food is more widely used to defend against environmental stress and replacing parts damaged cells due to contamination with toxic materials [11].

Heavy metals that present in waters will be processed to be deposit and will accumulate in life that exists in water through gills or through the food chain and ultimately to humans. This phenomenon is known as bioaccumulation or bio-magnification. Absorption in the network continues to increase in accordance with increase in lead concentration in the water and the long exposure of organism in contaminated waters. This is because the aquatic organisms are not able to regulate heavy metals Pb that enter the body of organisms [6].

Accumulation of heavy metals in fish body occurs because of a heavy metal that has entered into the body tend to form complex compounds with organic substances in the body. As a result, fixed metal formed tends to accumulate in the body of animal [12]. Heavy metals can also accumulate in the body tissues of other larger fish due to the food web as the fish eat the phytoplankton or smaller animal that contain heavy metals too. Hence, the concentration of heavy metals encountered higher in the animal's body with higher trophic level.
4. Conclusion
The results conclude the higher concentration of lead contained in the media, the higher concentration of lead can be accumulated by Oryzias javanicus. The ability of O. javanicus survive in lead contaminated waters to above the threshold of Pb with concentration more than 0.05 mg/L showed that the fish as a potential indicator organism.

References

[1] Darmono 1995  Environment and Pollution. Indonesia University Press. Jakarta. pp 36, 79-81, 130, 137-140.
[2] Johnson 1980. Aquatic Toxicity Test. Gajah Mada University Press. Yogyakarta.
[3] Ibrahim M. 2012. Uji lethal dose 50% [LD50] poliherbal [Curcuma xanthorriza, Rheinhobi hospita, Nigella sativa, Arcangelisia flava dan Ophiocephalus striatus] pada Heparmin terhadap Mencit [Mus musculus]. Res. and Dev. PT Royal Medicalink Pharmalab. 4-5.
[4] Tandjung 1982. Lead [Pb] Content and Ctenidia Microanatomy Structure and Digestive Gland of Anodontia woodiana Lea Origin from Serang Hilir Waduk Kedung Ombo. Bachelor Honour Thesis. Department of Biology FMIPA UNS. Surakarta.
[5] Darmono 2001. Review: Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. Analytica Chimica Acta, 606: 135–150.
[6] Alaerts, G and Santika SS 1987. Water Research Method. Usaha Nasional. Surabaya.
[7] Effendi 2003. Telaah Kualitas Air bagi Pengelolaan Sumber Daya dan Lingkungan Perairan. Skripsi. Faculty of Fishery and Marine Science. Agriculture Institute Bogor.
[8] Ercal N, Gurer-Orhan H, Aykin-Burns N 2001. Toxic metals and oxidative stress part I: Mechanisms involved in metal induced oxidative damage. Current Topics in Med. Chem. 1: 529–539.
[9] Sevcikova M, Modra H, Slaninova A and Svobodoya Z 2011. Metals as a cause of oxidative tress in fish: a review. Veterinarni Medicina 56 [11]:537-546.
[10] Yuniar V 2009. Toxicity of Mercury [Hg] on Survival Rate, Growth, Blood Profile and Damage Organ in Nile Fish Oreochromis niloticus. Bachelor Thesis. Faculty of Fishery and Marine Science. Bogor Agriculture Institute.
[11] Yosmaniar E, Supriyono and Sutrisno 2009. Lethal toxicity moluscicide niclosamide on juvenile of carp fish Cyprinus carpio. Res. J of Aquaculture. 4 [1]: 85-93.
[12] Riani E 2004. The effect of pollution on disable and extinction on marine organism. http://www.wwf.or.id/Default.php ID-570. Access 28 March 2005.