Acute toxicity test and LC50 value of mercury on *tubifex tubifex*

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**ABSTRACT.** Mercury is a heavy metal that has the highest toxicity. The toxicity of mercury will increase at the time of entry into the water. This study aims to determine the percentage of death Tubifex tubifex exposed to HgCl₂ and determine LC50-48 hours of mercury in *Tubifex tubifex* worms. The study was divided into two stages, preliminary test and definitive toxicity acute test (LC50-48 h). Concentrations are used to test toxicities on a logarithmic scale. Observations were made every 24 hours for 48 hours. Mercury was detected by Atomic Absorption Spectrophotometer (AAS) method. The results showed that the increase in *Tubifex tubifex* mortality was directly proportional to the increase in HgCl₂ concentration. The use of probit obtained LC50-48 h at a mercury concentration of 83.15 ± 3.89 μg/L, can be categorized into heavy metals with high toxicity. The tendency of toxicity decreases with the length of exposure time.

1. Introduction

The toxicity test aims to trace information about toxicity of substances in test organisms [1]. Toxicity tests were performed to determine the median lethal concentration (LC50) of the test animals [2]. Acute toxicity is an effect that can damage organs in the exposure of chemicals in a short time. In this study, acute toxicity was 50% deadly concentration of test animals (LC50). LC50 is an indicator of response resistance to metals [3].

Mercury is the cause of environmental damage. The entry of Hg into the environment usually comes from human activities [4]. Mercury includes heavy metals that are highly toxic. When entering into the body it will cause tissue damage and growth processes.

*Tubifex tubifex* includes widespread freshwater oligochaete, which lives in water/sediment. *Tubifex* sp. is an animal to be used as a bioindicator of organic water contamination. Some aquatic worms are found in wastewater treatment such as Tubificidae, Lumbriculidae and Aeolosomatidae. Tubificidae (eg *Tubifex tubifex*) has high consumption rates, long life spans and has the ability to tolerate pollutants and toxic compounds in conservation ecosystems [5]. Aquatic worms of the oligochaeta group are natural predators that have the ability to reduce mud [6]. But all water invertebrates including *Tubifex* worms can accumulate metals in their tissues [7]. In addition, *Tubifex tubifex* can be used in laboratory-scale toxicities assay [8].
This study aims to determine the percentage of *Tubifex tubifex* mortality exposed to HgCl$_2$ and determine LC$_{50}$-48 hours of mercury in *Tubifex tubifex*.

2. Methods

Research was conducted using experimental method. The test organism was the *Tubifex* worm that was cultivated. The mercury toxicity test was performed to determine the upper lethal limit and lower lethal limit for obtaining the median lethal concentration (LC$_{50}$).

In the acute toxicity test, the primary parameter was the percentage of *Tubifex tubifex* mortality for 48 h after exposure to HgCl$_2$. Supporting parameters measured were temperature, pH and dissolved oxygen.

The mercury toxicity test on *Tubifex tubifex* through two stages was preliminary and definitive test. The preliminary test was conducted for 24 hours to find the upper lethal threshold and lower lethality threshold. The upper lethal threshold concentration was the lowest concentration causing 100% mortality from the test animals, whereas the lower lethal threshold concentration was the highest concentration that did not cause death at all in the test animals [1].

The concentrations used for testing in preliminary tests were based on logarithmic scale [9]. The concentration of HgCl$_2$ used were: 1000 μg / L; 100 μg / L; 10 μg / L; 1 μg / L; 0.1 μg / L; 0 μg / L [1]. Observations on the preliminary test were the percentage of *Tubifex tubifex* deaths, observed every 24 hours for 48 hours of exposure. After the upper lethal threshold concentration and the lower lethal threshold are obtained then the concentration used in the definitive test is determined using the logarithmic formula. Determination of concentration was based on Quantitative Response by Finney [10]:

\[
\log \frac{N}{n} = k \times \log \frac{a}{n}
\]

\[
\frac{a}{n} = \frac{b}{a} = \frac{c}{b} = \frac{d}{c} = \frac{e}{d} = \frac{x}{e} \ldots = \frac{N}{x}
\]

Note :

N = Upper threshold concentration
n = Lower threshold concentration
a = The smallest concentration in the series of concentrations used
b = The b-concentration in the concentration series used
c = The c-concentration in the concentration series used
d = The d-concentration in the concentration series used
x = x concentration in the series of concentrations used
k = Number of interval concentrations tested

The definitive toxicity test aims to determine mercury toxicity (LC$_{50}$) during 48 hours exposure, observation every 24 hours for 48 hours. Determination of LC$_{50}$ value using probit analysis [11]. Probit analysis is used in toxicology to determine the relative toxicity of chemicals in living organisms. The probit analysis is done by looking at the response of the organism to various chemicals, then the each concentration is compared to get the result [12]. The relationship of logarithm values to the concentration of animal mortality test is in a linear function $y = a + bx$ [13, 14]. The LC$_{50}$ value is the concentration antiloge.

Measurement of water quality of the media was done at the time of observation. Water quality parameters were temperature (measured using thermometer), pH (determined using pH meter) and dissolved oxygen (determined using DO meter).

The results of acute toxicity test data were analyzed using probit analysis to determine the LC$_{50}$ value in exposure for 48 hours.
3. Result and Discussion

3.1. Preliminary test
The results obtained from the preliminary test were on the control of no Tubifex death due to no HgCl$_2$ exposure. At the highest concentration of HgCl$_2$ (1000 μg / L), the mortality of test animals was 100%, whereas in HgCl$_2$ exposure with different concentrations causing the mortality of test animals to be varied. Figure 1 shows Tubifex tubifex mortality increased in respond to increases concentrations of HgCl$_2$. Heavy metals such as mercury, cadmium and lead are toxic to aquatic animals [15]. Tubifex tubifex mortality in acute toxicity testing is due to the entry of mercury into the tissues of the body through the skin at the time of worming gas exchange. This is related to Tubifex tubifex nature that performs the process of breathing through the skin. Tubifex worms swallow sediment, selectively digest bacteria and absorb molecules through body walls [16].

![Figure 1. Tubifex tubifex mortality exposed to HgCl$_2$ for 24 hours on preliminary test](image)

3.2. Definitive Test (LC$_{50}$-48 hours)
The concentration on the definitive test was based on the upper threshold value and the lower threshold obtained in the preliminary test. Based on the analysis result, the treatment concentration as follows 15.85 μg / L; 25.12 μg / L; 38.81 μg / L; 63.09 μg / L; 99.98 μg / L. In a definitive acute toxicity test, the death of the test animal on the control does not exceed 20% which was the maximum possible mortality limit in the acute toxicity test [1]. Figure 2. shows that mortality on HgCl$_2$ exposure for 24 hours was lower than 48 hours exposure. The graph shows a tendency for increased mortality correspond to increasing exposure time. If the organism was exposed to heavy metals for long periods of time, more heavy metals will accumulated in the body of the organism. This relates to the nature of heavy metals that can not be decomposed, so that within certain periods, higher accumulation in the body of the organism. Mercury is one of the heavy metals whose toxicity rates are highest compared to other metals. Mercury is one of the most dangerous water pollutants due to its toxicity, bioaccumulative and non-biodegradable [17].

Based on probit analysis obtained LC$_{50}$-24 hour value achieved at HgCl$_2$ concentration 84.6 μg / L, while LC$_{50}$-48 hour equal to 83.15 ± 3.89 μg / L. However, the tendency of toxicity decreases with the length of exposure time. It is related to the process of adaptation of the organism to exposed heavy metals. Table 1 shows LC$_{10}$ to LC$_{99}$ on Tubifex tubifex exposed to HgCl$_2$ for 24 h and 48 h.
Figure 2. *Tubifex tubifex* mortality exposed to HgCl$_2$ for 24 h and 48 h on definitive acute tests.

Table 1. Lethal Concentration (LC) on exposure to HgCl$_2$ for 24 h and 48 h

| Lethal Concentration (LC) | Konsentrasi HgCl$_2$ (24 h) | Konsentrasi HgCl$_2$ (48 h) |
|---------------------------|-------------------------------|-------------------------------|
| 10                        | 12.71                         | 9.67                          |
| 20                        | 37.39                         | 34.89                         |
| 30                        | 55.18                         | 53.08                         |
| 40                        | 70.39                         | 68.63                         |
| 50                        | 84.6                          | 83.16                         |
| 60                        | 98.82                         | 97.68                         |
| 70                        | 114.02                        | 113.22                        |
| 80                        | 131.82                        | 131.41                        |
| 90                        | 156.49                        | 156.63                        |
| 99                        | 215.11                        | 216.53                        |

Figure 3 shows the probabilistic mortality chart of the *Tubifex* worm at HgCl$_2$ concentration level with 95% confidence interval (P > 0.5). LC$_{50}$-48 hours achieved at HgCl$_2$ concentration of 83.15 ± 3.89 μg / L. The graph shows that the increase in HgCl$_2$ concentration is directly proportional to the percentage of *Tubifex tubifex* mortality. The higher the concentration of HgCl$_2$, the number of *Tubifex tubifex* worms that die getting more and more. The percentage of deaths increased gradually correspond to increasing concentrations of heavy metals mercury chloride [18].

If the concentration of LC$_{50}$ is less than 1000 μg / L then the chemical is very toxic, whereas if the concentration of LC$_{50}$ between 1000 – 10000 μg / L is included in moderate category [19]. Based on these statements, mercury includes a highly toxic metal because within 48 hours, LC$_{50}$ achieved at exposure to HgCl$_2$ concentration of 83.15 ± 3.89 μg / L.
3.3. Water Quality Test Media

Table 2 shows that generally the water quality during the study at each treatment is still within the limits of the organism's life tolerance.

| Observation Time | pH       | Suhu (°C) | DO (mg/L) |
|------------------|----------|-----------|-----------|
| 24 h             | 7.8 – 8.4| 24-27     | 4.2-6.3   |
| 48 h             | 8.2 – 8.6| 25-29     | 4.1-6.7   |

Temperature may affect the presence and properties of heavy metals. Increased water temperatures tend to increase the accumulation and toxicity of heavy metals because of the high temperatures that will increase the rate of metabolism of organisms [20]. Tubifex worms are able to survive in the 20-29°C temperature range but the optimal temperature required for Tubifex worms is between 20-30°C. The degree of acidity (pH) of the medium was still within the tolerable range of Tubifex tubifex (7.8-8.6). Low pH values will affect the biochemical processes of waters such as nitrification process. The optimal pH range for Tubificidae is 6-8 [21].

4. Conclusion

The percentage of Tubifex tubifex mortality increased depend on the increasing concentration of HgCl₂. The value of lethal toxicity (LC50-48 hours) of mercury in Tubifex tubifex of 83.15 ± 3.89 μg / L can be categorized into heavy metal classes with high toxicity and a tendency to decrease toxicity with increasing length of exposure.

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