Toxicity Analysis of Effluent of Leachate Treatment Facility of Piyungan Landfill Using *Cyprinus carpio*

Any Juliari, Suphia Rahmawati, Alferina Julinda Grazella, Andik Yulianto, and Anja Asmarani

1Environmental Engineering, Universitas Islam Indonesia, Yogyakarta, Indonesia.
2Polytechnic of Marine and Fisheries, Sidoarjo, East Java, Indonesia

**Abstract.** Following regulation, Leachate Treatment Facility (LTF) was built in Piyungan landfill to reduce its negative impact to the environment. The effluent from this LTF is regularly monitored to check its compliance with government standard. However, the standard does not fully address the effect of combined components in a particular wastewater on living organism. Hence, it is necessary to conduct toxicity test to fill this gap. This study was aimed to analyze the acute toxicity status of leachate pretreatment and post-treatment from leachate treatment facility of Piyungan Landfill. The method used for the test is Whole Effluent Toxicity (WET) by using *Cyprinus carpio* as test organism. Acute toxicity test was performed by non-renewal static method for 96 hours. The result showed that LC50 of *Cyprinus carpio* are 1,633% and 8,740% for influent and effluent of LTF respectively. The LTF has reduced the Toxic Unit acute (TUa) of pretreated leachate with 61,246 in the influent to be 11,442 in the effluent. However, even in the effluent, the leachate is still classified as High Acute Toxicity. It is recommended to improve the performance of LTF to further reduce leachate toxicity level to be No Acute Toxicity.

1 Introduction

Piyungan Landfill is the major waste disposal site at Special Region of Yogyakarta which is located in Bantul District. The capacity of this landfill is 2.7 million m$^3$ of waste generating from Yogyakarta City, Sleman and Bantul Districts [1].

Landfill produces leachate. Leachate is the liquid that passes through or drains from a landfill. It is produced due to natural humidity and water infiltration in the covering layers and inner layers of landfill cells as the result of biological degradation of organic matter [2]. Acidic property of leachate enhanced the dissolution of hazardous substances such as heavy metal. Heavy metal content in leachate is one of major concern regarding leachate contamination to environment, especially to soil and groundwater. To prevent this problem to occur, landfill should be designed and managed in a way to isolate waste and its hazardous emission from environment.

* Corresponding author: any.juliani@uii.ac.id
In Indonesia, it has been regulated that one of the facilities should be present in a landfill is leachate treatment plant. Leachate should be treated to meet the effluent standard as stated in The Regulation of Ministry of Environment and Forestry No. 59/Menhk/Setjen/kum.1/7/2016. This effluent standard only covers physical and chemical parameters of pH, BOD, COD, TSS, Mercury, and Cadmium. It does not fully address the effect of combined components in leachate on living organism. On the other hand, leachate may contain other hazardous substances such as Cr, Cu, Pb, Ni and Zn which are also of environment and public health concern. Hence, it is necessary to conduct toxicity test to fill this gap. This study was aimed to analyze the acute toxicity status of leachate pre and post-treatment from leachate treatment facility (LTF) of Piyungan Landfill. The method used for the test is Whole Effluent Toxicity (WET) by using Cyprinus carpio as test organism. WET test result describes the toxic effect of an aqueous sample as measured by the response of organisms upon exposure to that particular sample [3]. Cyprinus carpio was selected as testing organism because of its high economic value and high sensitivity to pollutants. It is also a commonly used fish aquatic organism for toxicity test worldwide. The result of this study serves as performance evaluation of leachate treatment facility as well as to provide data for the development of effluent standard which addresses toxicity aspect.

2 Methods

2.1 Sampling

Leachate sampling procedure refers to Indonesian National Standard (SNI 6989.59, 2008 on Water and Wastewater, section 59: Waste Water Sampling Method). The samples were taken from inlet and outlet of LTF of Piyungan Landfill. Characteristic of influent leachate as measured by two times of sampling is presented in Table 1.

| No | Parameters | Unit | Influent 1 | Influent 2 | Method |
|----|------------|------|------------|------------|--------|
| 1  | pH         | -    | 6.4        | 6.5        | SNI 06-6989.11-2004 |
| 2  | BOD        | mg/L | 253.93     | 55.77      | SNI 6989.72-2009 |
| 3  | COD        | mg/L | 2274.29    | 2115.00    | SNI 06-6989.2-2004 |
| 4  | BOD/COD    |      | 0.11       | 0.03       | - |
| 5  | TSS        | mg/L | 1138.00    | 568.33     | SNI 6989.72-2009 |
| 6  | N Total    | mg/L | 1828.26    | 3068.48    | SK SNI M-47-1990-03 |
| 7  | Cadmium    | mg/L | 0.223      | 0.218      | SNI 6989.16-2009 |

2.2 Acclimatization

Organisms for toxicity testing should be approximately at the same age and taken from the same source to minimize the diversity of response to tested substances. Fingerlings of Cyprinus carpio at the age of around 35 days and size ranges from 3-5 cm were selected for this test [4]. Fingerlings are fully developed, entirely covered with scales (with the exception of fish that have no scales), and appear just like adult fish. Despite their adult appearance, the immune system of fingerlings is still developing. Adaptive immune competency occurs roughly after the age of two months [5].

A 7 days acclimatization process allows fish to adapt to a new environment prior to actual test [6]. During the process, fish were fed with nutrient 3% of their body weight on...
daily basis [7]. According to [8], water quality requirement during acclimatization is presented in Table 2.

| Parameters                  | Unit | Value |
|-----------------------------|------|-------|
| Temperature                 | °C   | 25-30 |
| pH                          | -    | 6.5-8.5 |
| Dissolved Oxygen/DO         | mg/L | Minimum 5 |

Table 2. Water quality for acclimatization

The water used during this process should not exceed 14 days because it allows the growth of bacteria, fungi, or algae [9]. Fish should be in healthy condition, no observed signs of for actual test process. The mortality of fish during acclimatization should be less than 10 % to allow for toxicity testing. Once exceed, acclimatization process should be repeated by using a new batch of fishes [10].

2.3 Initial Test

The initial test was performed to determine appropriate concentrations for toxicity test [6]. After acclimatization, the fish were transferred to toxicity test reactors with variation of leachate concentration of 0%; 6.25%; 12.5%; 25%; 50%; and 100% respectively [11]. It was applied both for influent and effluent of LTF of Piyungan, so there were 2 series of 6 comprised test reactors. The 0% concentration served as control reactor [9].

Each reactor was filled with 10 liters of sample solution and 20 testing fishes. The density factor of 1 or 2 fish(es) per liters of sample solution did not give significant difference to the survival rate of *Cyprinus carpio*. This density could still allow a proper condition to fulfill nutritional and space needs of this organism [12].

2.4 Actual Test

The actual test was conducted to determine Lethal Concentration 50 or LC$_{50}$ which is defined as the concentration of testing sample that kills half (50%) of organisms within a specified period of time. There are four methods available to determine LC50, namely Graphical, Spearman-Karber, Trimmed Spearman-Karber, or Probit Method [13]. Selection of which method to use is based on mortality data during the initial test. The duration of the test may vary from 24 hours to 96 hours depending on the objectivity of the test and requirement of relevant standard or regulation. The test results are acceptable if at least 90 % of the fish survive in the control reactor [10]. Low dissolved oxygen (DO) concentrations may give impact on the test results. Then aeration can be performed in all test and control reactors [9]. The concentration used for actual toxicity test depends on that from the initial test. The suggested dilution factor for this stage is 0.5 [11]. The value of LC50 is then calculated by using EPA Probit Analysis Program Software for Calculation of LC/EC Value Version 1,5.

2.5 Toxicity Classification

Toxic Unit (TU) is a measure of toxicity in an effluent as determined by the acute toxicity units (TUa) or chronic toxicity units (TUc) measured. The higher the TU, the greater the toxicity. Toxic Unit - Acute (TUa) is 100 times the reciprocal of the effluent concentration that causes 50% of the organisms to die in an acute toxicity test [14]. The Classification
based on the value of TUa can be seen in table 2.3 and it can be derived by using equation (1)

\[ TUa = \frac{100}{LC_{50}} \] (1)

TUa = Toxic Unit acute
LC_{50} = Concentration to cause the death of 50% of population

### Table 3. Classification Based on Toxicity Assessment [15]

| No | Kelas     | Tingkat Toksisitas      | Toxic Unit |
|----|-----------|-------------------------|------------|
| 1  | Class I   | No acute toxicity       | <1         |
| 2  | Class II  | Significant acute toxicity | 1-10      |
| 3  | Class III | High acute toxicity     | 10-100     |
| 4  | Class IV  | Very high acute toxicity | >100       |

### 3 Result and Discussion

#### 3.1 Acclimatization

During acclimatization, the mortality of *Cyprinus carpio* is 25 of 450 fishes or 5.56% and 5 of 450 or 1.11%. As it was under 10%, acclimatized fish can be used for toxicity tests.

#### 3.2 Initial Test

Sample from influent of LTF caused 100% mortality of *Cyprinus carpio* in all concentrations which occurred after 1-hour exposure of fishes in testing reactors. There was no mortality of fish in the control reactor, so the data from this initial test is acceptable. Based on data from this test, the range of concentrations for actual test is between 0-6.25%. As suggested, with dilution factor of 0.5, leachate concentration used for actual test were 0%; 0.20%; 0.39%; 0.78%; 1.56%; and 3.13%.

On the other hand, sample from effluent of LTF caused 100% mortality of *Cyprinus carpio* in the reactor of 50% concentration of leachate within 1 hour and within 24 hours in the reactor of 25% concentration of leachate. No mortality detected at the control reactor, so the data is acceptable. Based on this data, concentrations for toxicity tests ranged between 0-25% concentration of leachate. With dilution factor of 0.5, the concentrations used for toxicity test were 0%; 0.78%; 1.56%; 3.13%; 6.25%; and 12.5%.

#### 3.3 Actual Test

The profile of mortality data during the actual test for influent as well as the effluent of LTF of Piyungan are presented in Figure 3.1 and 3.2. Within the period of 24 hours, for the influent sample, the concentration of 3.13% caused 100% mortality. While at the lower concentration of 1.56%, only 40% fishes were killed within the same period of time. For the effluent sample, 60% fishes were dead in the reactor of 12.5% concentration within 24 hours. The figure is decreasing for the reactors with lower concentration.

These figures show that within the same period of time, the influent of LTF caused more death with lower concentration than that from the effluent of LTF. In other words, mortality of *Cyprinus carpio* is higher in influent comparing to that in effluent of LTF.
By using the EPA Probit Analysis Program Software for Calculation of LC/EC Value Version 1.5, the value of LC$_{50}$ for influent and effluent of LTF are 1,633% and 8,740% respectively. This means that the 1,633% concentration of influent sample killed 50% of fishes within 24 hours while it takes a higher concentration of 8,740% for effluent to kill the same number of fish. A study conducted by Alkassasbeh, et.al [16] presented the value of LC$_{50}$ of leachate from three different landfills in Malaysia by using the same testing organism (Cyprinus carpio) was found to be 1,132; 2.0 and 3.822 %. The leachate from these three landfills has BOD values of 1228, 866 and 507 and COD values of 7600, 3733 and 1640 respectively. Comparing to that study, with relatively lower BOD and COD value, leachate from Piyungan Landfill has lower LC$_{50}$ which means higher toxicity. The ratio of BOD to COD can be the cause of this state. Samudro et.al. stated that the BOD/COD ratio of less than 0.1 is considered to be within a toxic zone [17]. This value indicates the presence of large portions of low- biodegradability organics such as humic and fulvic acids.
The value of LC$_{50}$ was then used to determine the Toxic Unit acute (TUa) for influent and effluent of LTF. The higher the value of TUa the more toxic one testing sample would be. The value of TUa for influent and effluent of LTF is presented in Table 4.

**Table 4.** LC$_{50}$ and TUa value of influent and effluent sample

| No. | Sample  | LC$_{50}$ | TUa  | Classification        |
|-----|---------|-----------|------|-----------------------|
| 1   | Influent| 1,633     | 61,246 | High acute toxicity   |
| 2   | Effluent| 8,740     | 11,442| High acute toxicity   |

According to their TUa value, both are classified as high acute toxicity. However, the value for influent is much higher than that of effluent. So that influent of LTF is more toxic than its effluent. The treatment process in LTF has reduced the toxicity level of leachate. This is supported by the data of laboratory analysis of parameters regulated by government standard as stated in the Regulation of Ministry of Environment and Forestry No 59/Menhk/Setjen/kum.1/7/2016. In general, the value of all 6 parameters in the effluent are lower than those in the influent of LTF but still above the standard. Most concern is put to the parameter of Cadmium which is classified as heavy metal. Cadmium is considered to be the second most hazardous heavy metal to the environment. It is biologically reactive with acute and chronical toxicity [18]. Maximum concentration of cadmium for fish is 0,001 mg/L [19].

Apart from cadmium, other toxic heavy metal substances which are not regulated may not be treated well and also exceed the standard. This may contribute to the still toxic state of leachate after treatment.

**Table 5.** Laboratory analysis of influent and effluent of LTF

| No | Parameter | Unit | Influent of LTF | Effluent of LTF | Standard* |
|----|-----------|------|-----------------|-----------------|-----------|
|    |           |      | A               | B               | A         | B         |           |
| 1  | pH        | -    | 6,4             | 6,5             | 6,5       | 6,4       | 6-9       |
| 2  | BOD       | mg/L | 253,93          | 55,77           | 15,01     | 28,96     | 150       |
| 3  | COD       | mg/L | 2274,29         | 2115,00         | 550,71    | 524,58    | 300       |
| 4  | TSS       | mg/L | 1138,00         | 568,33          | 120,00    | 466,67    | 100       |
| 5  | N Total   | mg/L | 1828,26         | 3068,48         | 1119,57   | 1828,26   | 60        |
| 6  | Cadmium   | mg/L | 0,2229          | 0,2180          | 0,1747    | 0,1615    | 0,1       |

* Regulation of Ministry of Environment and Forestry No 59/Menhk/Setjen/kum.1/7/2016

**3.4 Physical Appearance of Testing Fish**

Exposure to leachate had changed the physical appearance of testing fishes especially at the colors of the gills and body in general. The change of gills into paler color is caused by the cessation of blood circulation from the gills of the fish. The cessation of blood circulation can be caused by the excess of mucus production, lack of oxygen, and gill blockage by suspended particles [19]. Moreover, the dead fish has thick mucus and pale body color. Excess mucus production and discoloration of fish showed ammonia and heavy metal poisonings [19].
4 Conclusion

Leachate of Piyungan Landfill is classified as High Acute Toxicity Level both for influent and effluent of its leachate treatment facility. The LC 50% of *Cyprinus carpio* for influent sample is 1,633% with Toxic Unit acute (TUa) of 61,246 and for effluent sample is 8,740% with Toxic Unit acute (TUa) of 11,442.

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