Acute Toxicity of domestic landfill leachate to carp fish 
(Cyprinus carpio)

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Abstract. Leachate has the potential to pollute rivers because it contains high organic and inorganic materials. Acute toxicity to fish may occur in the short term. This study aims to determine the effect of acute leachate toxicity on mortality and histopathological features of carp gill organs. This study used the Completely Randomized Design (CRD) used five leachate treatments (0.5%; 1%; 1.5%; 2% and 2.5%) with four replications. Each treatment used ten fish. The results showed that the higher the leachate concentration, the higher the mortality of carp. Even mortality reached 100% occurred in groups of fish exposed to 2.5% leachate for 96 hours. Histopathological changes in carp gill organs showed hyperplasia, hypertrophy, edema, and congestion due to exposure to leachate.

1. Introduction
The final disposal site (TPA) is one of the most widely practiced methods for waste disposal in congested cities because 95% of the total waste is collected and disposed of in TPA [1]. Most of the waste management in Indonesia still relies on TPA. The majority of landfills in Indonesia are open dumping landfill types [2]. The application of an open dumping system causes the spread of leachate due to the absence of a retaining layer under the surface of the waste pile [3].

Leachate is a liquid resulting from the seepage of water through piles of garbage [4]. Leachate has the potential to pollute rivers because it contains high levels of heavy metals and ammonia [5, 6]. The mercury content detected in the leachate of the Benowo TPA was 2.66 mg/l [7]) and the ammonia content was 16,207 mg/l [8]. High levels of ammonia can harm aquatic organisms and cause eutrophication and depletion of dissolved oxygen (DO) [9]. During ammonia intoxication from water, ammonia can penetrate into the blood and tissues through the gills and skin or can accumulate in organisms to toxic concentrations [10].

Several studies have been conducted to determine the impact of landfill leachate toxicity on aquatic organisms. Alkassasbeh et al. [1] using C. carpio, and Emenike et al. [11] used the fish Orechromis mossambicus. Olujimi et al. [12] showed gill and liver damage based on histopathological analysis of African catfish (Clarias gariepinus) that survived after exposure to leachate. Oliveira et al. [13] evaluated the effect of domestic landfill leachate on the gills and digestive glands of the bivalves Corbicula fluminea. Carabalí-Rivera et al. [14] used Daphnia pulex and Poecilia reticulata as biological indicators to evaluate the potential for leachate toxicity, while Kartikasari et al. [15] using Daphnia sp. Žaltauskaitė and Vaitonytė [16] examined the toxicity of leachate and surface water of municipal solid waste landfills using bioassays with aquatic plants (Lemna minor L.) and micro-
invertebrates (*D. magna*). Emenike *et al.* [5] evaluated the toxicological impact by observing the gill histology of *Pangasius sutchi* and *C. batrachus* as indicator organisms of leachate pollution in waters. This study was conducted to evaluate the risk impact of Ngipik landfill leachate using *C. caprio* as a test animal in an acute toxicity test. TPA Ngipik is one of the large-scale landfills that has been operating in Gresik Regency since 2003 with an open dumping management system [17]. Mortality and histopathological changes of carp (*C. carpio*) gill organs were used to assess the level of toxicity of Ngipik landfill leachate.

2. Materials and methods

2.1 Materials

The material used in this study was carp (*C. carpio*) with a size of 3-4 cm and a weight of <3 g [18]. Carp fish are obtained from the Gunung Sari fish market. The leachate used comes from the Ngipik Gresik TPA and has a dark black color. Before use, the leachate is filtered to reduce the amount of sludge and waste. Materials for the preparation of organ histopathology preparations include fixative solutions, aquadest, formalin, liquid paraffin, alcohol 70%, 80%, 85%, 90%, 95%, and absolute alcohol, xylene, acid alcohol, and dye hematoxyline eosin (HE).

2.2 Methods

The aquarium (30 x 20 x 25) cm$^3$ was cleaned with detergent, rinsed, and disinfected with a dose of 1 ppm chlorine, and dried in the sun. Each aquarium was filled with 10 test fish. The acclimatization process of the test fish was carried out for seven days.

This experimental study used a completely randomized design (CRD) consisting of five treatments with four replications. The number of treatments was five concentrations of leachate which were determined based on the upper and lower threshold values [19] referring to [20]:

\[
\log \frac{N}{n} = K \log \left( \frac{a}{n} \right) \\
\frac{a - b}{n} = c \quad d = e
\]

Description: $N =$ Upper threshold concentration, $n =$ Lower threshold concentration, $K =$ Amount of test concentration, $a =$ The smallest test concentration, $b, c, d, e =$ The desired test concentration

The results of the preliminary test observation for 24 hours on carp (*C. carpio*) obtained an upper threshold concentration of 3% and a lower threshold concentration of 0%. Based on the results of calculations using this formula, this study used five treatments of leachate concentration (0.5%, 1%, 1.5%, 2%, and 2.5%).

Substitution of media water and feeding of fish were not carried out during the treatment. Observation of leachate toxicity was based on fish mortality after exposure to leachate for 6 hours, 24 hours, 48 hours, 72 hours, and 96 hours.

The main parameters of the study were mortality and histopathological features of carp gills. The mortality of the test fish was observed after exposure to leachate at the 6th, 24th, 48th, 72nd, and 96th hours. Histopathological changes of gill tissue were observed at the end of the study, namely at 96 hours. Histopathological changes in gill tissue were classified based on scores of 0, 1, 2, 3, and 4 [21]. Score 0 if there are no histopathological changes. Score 1 if in one field of view there is tissue damage <25%. Score 2 if there are 26-50% damaged tissue, score 3 if there are 51-75% damaged tissue, and score 4 if there are > 76-100% damaged tissue in one field of view. The scoring data were analyzed with the Kruskal Wallis test and then continued with the Mann Whitney test.
The supporting parameters of the research were the physical and chemical properties of the Ngipik TPA leachate which were analyzed at the Balai Riset dan Standardisasi Industri, Surabaya, East Java - Indonesia. The water quality parameters measured at the beginning and end of the study included temperature with a thermometer, pH with a pH meter, dissolved oxygen (DO) with a DO test kit, and ammonia with an ammonia test kit.

3. Results and discussion

3.1 Results

3.1.1 Characterization of Ngipik Landfill Leachate

The physical and chemical properties of the Ngipik TPA leachate, the results of the 2014 analysis show that the COD, TSS, and mercury values exceed the leachate quality standards according to the 2016 Minister of Environment and Forestry regulation (Table 1).

| Parameter                        | Value   | Leachate Quality Standards* |
|----------------------------------|---------|-----------------------------|
| pH                               | 7.9     | 6-9                         |
| BOD                              | 125 mg/l| 150 mg/l                    |
| COD                              | 3936 mg/l| 300 mg/l                   |
| Ammonia free (NH3-N)             | 129.7 mg/l | -                         |
| Total Suspended Solids (TSS)     | 1890 mg/l| 100 mg/l                    |
| Total dissolved solids (TDS)     | 7600 mg/l| -                           |
| Timbal (Pb)                      | 0.33 mg/l| -                           |
| Merkuri (Hg)                     | 0.022 mg/l| 0.005 mg/l                  |

*Regulation of the Minister of Environment and Forestry of the Republic of Indonesia Number P.59/Menhk/Setjen/Kum.1/7/2016

3.1.2 Leachate toxicity to carp fish

The increase in fish mortality occurred in all treatments along with the length of exposure to leachate (Table 2). Mortality of 67.5% and 100% occurred in the group of carp exposed to leachate concentrations of 0.5% and 2.5% for 96 hours, respectively. This was because the concentration of 2.5% leachate contained higher ammonia (8.75 mg/l) than the concentration of 0.5% leachate (3 mg/l) (Table 3).

Table 2. Data on the percentage (%) of mortality of carp (C. carpio) after exposure to Ngipik landfill leachate

| Exposure time | Mortality percentage (%) | P1 | P2 | P3 | P4 | P5 |
|---------------|--------------------------|----|----|----|----|----|
| 6             | 0                        | 2.5| 10 | 12.5| 30 |
| 24            | 0                        | 2.5| 25 | 50 | 60 |
| 48            | 17.5                     | 20 | 70 | 72.5| 77.5|
| 72            | 40                       | 72.5| 85 | 85 | 95 |
| 96            | 67.5                     | 85 | 90 | 95 | 100|

Description: P1= 0.5% leachate, P2= 1% leachate, P3= 1.5% leachate, P4= 2% leachate and P5= 2.5% leachate.
Table 3. The average value of water quality at the beginning and end of the study

| Leachate Concentration (%) | Temperature (°C) | DO (mg/l) | pH | Ammonia (mg/l) | Temperature (°C) | DO (mg/l) | pH | Ammonia (mg/l) |
|----------------------------|------------------|----------|----|---------------|------------------|----------|----|---------------|
| 0.5%                       | 28.5             | 4        | 7  | 3             | 29               | 4        | 7  | 3             |
| 1%                         | 29               | 3.5      | 7  | 3             | 29               | 4        | 7  | 3             |
| 1.5%                       | 29               | 4        | 7  | 3             | 28.25            | 3.5      | 7  | 5             |
| 2%                         | 28.25            | 4        | 7  | 6.25          | 28.5             | 4        | 7  | 6.25          |
| 2.5%                       | 28.5             | 3.5      | 7  | 8.6           | 29               | 3.5      | 7  | 8.75          |

3.1.3 Histopathological changes in gills
Edema in the primary lamellae and secondary lamellae occurred at P5 (2.5% leachate concentration) while P2 (1% leachate concentration) experienced edema in the secondary lamellae. At P4 (2% concentration) and P3 (leachate concentration 1.5%), the secondary lamellae were hyperplasia (Figure 1). It is characterized by the widening of the secondary lamella which causes the secondary lamella to appear fused. The leachate concentration treatment had a significant effect \( (p<0.05) \) on the scoring of the histopathological picture of carp gills. The 2.5% leachate treatment had the highest average score and was significantly different \( (p<0.05) \) with the 2%, 1.5%, 1%, and 0.5% leachate treatment (Table 4).

Table 4. The histopathological scoring of carp fish gills

| Leachate Concentration (%) | Average Score |
|----------------------------|---------------|
| 0.5                       | 1.25±0.43a    |
| 1                         | 2.25±0.43c    |
| 1.5                       | 2.5±0.5bc     |
| 2                         | 3±0b          |
| 2.5                       | 3.75±0.43a    |

Description: Different lowercase letters in the same column for each test parameter indicate a significant difference at the significance level \( (\alpha = 0.05) \).
3.2 Discussion

The BOD value of the Ngipik TPA leachate is 125 mg/l with a BOD/L ratio. COD is 0.03. According to Kjeldsen [22], a BOD/COD ratio value of less than 0.1 indicates that the landfill is old and stable. If the BOD/COD ratio > 0.5 indicates the TPA is young, while the ratio 0.1-0.5 indicates a stable portion of the leachate. Emenike et al. [11] argue that the levels of BOD and COD are a reflection of the high concentration of degradable organic matter in the waste stream. Likewise, the high concentration of total dissolved solids (TDS) results from the deposition of waste in the landfill which contains a high concentration of dissolved organic matter. The high concentration of NH3-N in TPA Ngipik Gresik, namely 129.7 mg/l, contributed significantly to the acute toxicity of carp. According to Alkassasbeh et al. [1], nitrogen ammonia (NH3-N) in landfill leachate is the result of biological degradation of amino acids and nitrogenous organic matter. The pH value of the Ngipik landfill leachate is 7.9. The pH value leading to number 8 indicates that the liquid waste contains a lot of ammonia (NH3) [23].

Carp fish exposed to Ngipik TPA leachate changed their behavior. Significant behavioral changes such as difficulty breathing and the tendency of fish to gather on the water surface occurred at concentrations of 2% and 2.5% until 95% and 100% mortality occurred at 96 hours while concentrations of 1.5%, 1%, and 0.5% only experienced changes in color and mucosa excessive and 100% mortality did not occur at the 96th hour. Alkassasbeh et al. [1] reported that carp fish exposed to leachate with different concentrations loss balance, difficulty breathing, excessive mucosal secretions, and gathered on the surface to breathe. Emenike et al. [11] reported that the mortality rate of *O. mossambicus* increased with increasing concentrations of leachate containing potentially toxic components.

Ammonia toxicity (96 h LC50) for freshwater fish species is in the range of 0.0068-2.0 mg/l NH3 [24]. Devaraj et al. [25] argue that ammonia can have a severe toxic effect on gills in direct contact with the aquatic environment. Damage to the gills will affect the vital functions of taking oxygen, and
excreting carbon dioxide and ammonia. According to Schaperclaus [26], fish exposed to acute toxic ammonia have quite a lot of mucus secretion, swelling of the epidermis, and hyperemia all over the body surface or only on the gills. The gills also show hyperplasia and at an advanced stage, bleeding, histolysis, and necrosis occur.

Hyperplasia causes proliferation of adjacent lamellae cells, reducing the inter-lamella space, resulting in fusion of the lamellae [27]. Carvalho et al. [28] suggest secondary lamellar fusion is likely due to severe lamellar hyperplasia leading to partial or total fusion of lamellar capillaries within a hyperplastic epithelial mass. According to Santos et al. [27], damage to gill tissue can be a gill defense mechanism against stressors in the polluted aquatic environment.

The hypertrophy that occurred in the first treatment (leachate concentration 0.5%) was seen from the enlargement of the secondary lamella cells without increasing the number so that they looked thick. Hypertrophy is caused by suspended matter and dissolved material from the Ngipik landfill leachate, which causes the gill organs to become irritated and secrete mucus as a form of protection. According to Sukarni et al. [29], mucus secretion can cover the surface of the gill lamella, which causes the exchange of O2 with CO2 to be inhibited so that there is no binding of oxygen by blood hemoglobin. As a result, oxygen transportation throughout the body is not smooth, causing congestion. According to Ferguson [30], congestion occurs when the gills experience damming of blood in the primary lamella, which is marked by an accumulation of red blood cells in the blood vessels.

4. Conclusion

This study proves that leachate from the Ngipik TPA has a toxic effect on carp (C. carpio). The level of mortality and the severity of histopathological changes depended on the concentration of leachate with a duration of exposure of 96 hours. The toxic effects of leachate on carp fish can provide information to support better waste management.

5. References

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