

Characterization and toxicological evaluation of leachate from Bacolod city sanitary landfill using tilapia (*Oreochromis niloticus*): a preliminary study

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Abstract. This study was carried out to characterize and assess the toxicity of Bacolod City Landfill leachate using Tilapia as bio-indicator species. Acclimatized *Oreochromis niloticus* fishes (length of 14 cm \pm 2.0 cm and weight of 11.8 g \pm 0.9 g) were introduced into each treatment tank containing three different concentrations of leachate in duplicates (10 ppm, 50 ppm and 100 ppm) and a control group. Physical reactions were observed such as erratic swimming, loss of reflex, hyperactivities, and surfacing. Reactions and mortality rate increased with increasing concentration and duration of exposure. Mortality rate using One-way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) revealed a significant difference between treatment groups and exhibited 100 ppm as the most potent concentration. Heavy metal analysis of Cu and Pb as well as physicochemical analysis of color, Chimecal Oxygen Demande (COD) and Total dissolved Solids (TDS) exceeded the country's environmental standard set by DENR. The 96 h bioassay stemmed the median lethal concentration (LC50) at 57.688 ppm. The study provides a biomarker database in taking action for the responsible enactment and enforcement of laws to upgrade, enhance and install framework for proper treatment of sanitary landfill leachate in cities and municipalities in the country.

1 Introduction

The anthropogenic activities of humans produce wastes which directly contribute to the contamination of several bodies of water. The effect of leachate from waste dump sites percolating into nearby water bodies could have adverse effects on the aquatic organisms particularly fish [1]. This suggests that solid wastes may be risky to life if not properly managed and have elicited strong international concerns about the possible environmental and health effects of living organisms in the vicinity of the wastes. Toxicity assessment with bioassay method has been widely used to gage landfill leachate toxicity. It has been used by several researchers worldwide to calculate the amount of substance an organism can be exposed to before adverse effects are observed [2].

Many organisms were used as biological indicators to assess the toxicity of landfill leachate such as bacteria, green algae, fish, and others. In the Philippines, the most common Tilapia species found is Nile Tilapia (*Oreochromis niloticus*) and Black Tilapia (*Oreochromis mossambicus*). All these Tilapias are consumed by Filipinos as food. To cater the fish demand supply, Tilapias are bred in a large scale. These fishes are reared in ponds, cages or pens and they grow well in fresh water and brackish waters. As with other types of Tilapia fishes, *O. niloticus* are

omnivorous feeder in which it can clean up any edible garbage in rivers and in any drainage or irrigation canals. It is highly adaptable and is an easily cultured type of fish [3].

Due to its characteristic as omnivore's feeder, this fish has the potential to get contaminated from various types of pollutants and it can cause toxicity to the food chain. In addition, *O. niloticus* can be one of the indicators for fish bioassay to determine the toxicity of leachate. The fish is a type of freshwater fish that can be found in rivers and lakes. For the supply purpose, many entrepreneurs used to seed the fish in the fish farm to cover the market demand. However, for people in the rural areas or those who likes fishing, they used to catch the fish in the lake or river to be consumed as a food. A study by Stauber (2000) has determined heavy metal accumulation in Tilapia sp. Fish [4].

The study showed that Tilapia sp. in the lake or river has the potential to be polluted with leachate and the safety of this fish for human consumption is dubious. Very limited research is found dealing with *Oreochromis niloticus*. Therefore, this study was carried out to characterize the quality of leachate of the Bacolod City Sanitary Landfill, assess its toxicity using *O. niloticus* and describe the behavioral and physiological responses when exposed to 96 h toxicity bioassay in varying leachate concentrations.

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2 Methodology

This section discusses the research design, method, and procedure for the investigation of the ecotoxicological effects of Bacolod City Solid Waste Landfill leachate on *Tilapia (Oreochromis niloticus)*. This also provides the statistical treatment used for data analysis.

2.1 Research Design

In view of the nature of the specific objectives of this research study, the study employed experimental research design. It is the blueprint of the procedure that enables researchers to test the hypotheses by reaching valid conclusions about relationships between independent and dependent variables. This type of research methodology is a fact-finding procedure that is primarily concerned with the experimental data and information about the characterization and toxicological evaluation of leachate from Bacolod City Sanitary Landfill using *Oreochromis niloticus*.

2.2 Sampling Site

Bacolod City, a highly urbanized city and the capital of the Province of Negros Occidental located on the northwestern coast of Negros Occidental is bounded on the northwest of the City of Talisay, on the east and southwest by the City of Bago and on the West by Guimaras Strait. According to the Philippine Statistics Authority, as of 2015, Bacolod City has a population of 561,875 with 61 barangays and has a total land area of 15,610 hectares. It occupies 1.97% of the total area of the province. With geographic coordinates of 10.6407°N, 122.9690°E, the city generates about 1,200 cubic meters of wastes/day.

2.3 Leachate Sampling

Raw landfill leachate was collected from Bacolod City Solid Waste Landfill leachate discharge point through grab sampling method in a clean, double-stoppered polyethylene bottles, properly labelled and sealed. It was immediately transported and stored to the laboratory on ice (4°C) until use 48 h later to prevent chemical degradation for laboratory analyses.

2.4 Physicochemical Analysis

Physicochemical analysis of the leachate was performed in the Negros Prawn Producers Cooperative Analytical and Diagnostic Laboratory following the APHA Method 4500-F: Standard Methods for the Examination of Water and Wastewater (AHA-AWWA) of the American Public Health Association, 2005 on color, pH, chemical oxygen demand, total suspended solids, and total dissolved solids.

The results of the physicochemical analysis were compared to the Department Administrative Order 2016-08-WQG (Water Quality Guidelines) and General Effluent Standards (GES) which was approved

on May 24, 2016 by the Department of Environment and Natural Resources.

2.5 Heavy Metal Analysis

Heavy metal contents including Pb, Cd, Cu, and Zn were measured by Flame Atomic Absorption Spectrometry (FAAS). The results of the heavy metal analysis were compared to the Department Administrative Order 2016-08-WQG (Water Quality Guidelines) and General Effluent Standards (GES) of the Department of Environment and Natural Resources [5].

2.6 Fish Collection and Acclimatization

Eighty *Tilapia (Oreochromis niloticus)* fishes with a total average length of 14 cm \pm 2.0 cm and mean weight of 11.8 g \pm 0.9 g was utilized for the investigation. These were provided by the Office of the Provincial Agriculturist of the Province of Negros Occidental and were sent to the fisheries division for validation and authentication.

The care and use of these fishes were in accordance with the international guidelines on the use of fishes for research (American Fisheries Society, 2004). They were acclimatized and maintained in a glass tank (30 L capacity) containing well aerated borehole water at 26 \pm 2°C for 14 days.

Water in the glass tank was changed at three days' interval to prevent the build-up of metabolic wastes. The fishes were fed twice daily with fish meal at 3% body weight [6]. The natural photoperiod following the 12 h dark and light cycles was observed. Feeding was discontinued 24 hours prior to the beginning of the experiment.

2.7 Toxicity Testing of Raw Leachate

Acute toxicity test was carried out to ten acclimated *Oreochromis niloticus* that were introduced into each treatment tank containing three different concentration of leachate in duplicates (10 ppm, 50 ppm, 100 ppm) and a control group. The toxicity test was based on the biostatics protocol recommended by EPA ECO (2008) [7].

The fishes in the test and control tank were observed for abnormal behavior/physiological and mortality within 96 h of exposure. A fish was considered dead when there was lack of opercula movement when prodded with a glass probe [8]. Results were encoded and a computer software was employed for the analysis of the data based on the set objectives of the study.

3 Results and Discussions

This chapter deals with the presentation, analysis, and interpretation of data from the research design and specific objectives of the study.

3.1 Physicochemical Analysis Results

Table 1 illustrates the physicochemical analysis of the leachate sample collected at Bacolod City landfill and evaluated using Standard Method for the Examination of Wastewater and APHA-AWWA. Color, COD and TDS surpassed the permissible limit set by DENR.

Table 1. Physicochemical Analysis of the Bacolod City Solid Waste Landfill Leachate vs DENR standards.

Parameters	Results Obtained	
	Bacolod City	DENR
pH	8.80	6.5-9.0
Color	1,280 TCU	150 TCU
Chemical Oxygen Demand (COD)	22,994.4 mg/L	100 mg/L
Total Suspended Solids (TSS)	79 mg/L	100 mg/L
Total Dissolved Solids (TDS)	5,589 mg/L	1,000 mg/L

3.2 Heavy Metal Analysis Results

Table 2 shows the heavy metal analysis of Cu, Zn, Cd and Pb on leachate sample of the Bacolod City Sanitary Landfill using Flame Atomic Absorption Spectrometry method vs DENR standards. Copper and lead exceeded the maximum standard of the country's environmental regulatory organization.

Table 2. Heavy Metal Analysis of the Leachate Sample vs DENR Standards.

Heavy Metal Parameters	Results Obtained	
	Bacolod City	DENR
Copper (Cu)	0.150 mg/L	0.04 mg/L
Zinc (Zn)	0.593 mg/L	4 mg/L
Cadmium (Cd)	<0.002 mg/L	0.01 mg/L
Lead (Pb)	0.218 mg/L	0.1 mg/L

3.3 Behavioral and Physiological Responses

Table 3 shows the behavioral reactions of *O. niloticus* within the 96 h toxicity exposure to Bacolod City Solid Waste Landfill Leachate. Fishes exposed to leachate showed irregular swimming, loss of reflex, hyperventilation, surfacing changes in behavior and increasing opercula aeration and movement.

Table 3. Behavioral Responses of *O. niloticus* during 96 h Exposure to Bacolod City Landfill Leachate.

Behavior	Concentration			
	Control	10 ppm	50 ppm	100 ppm
Erratic Swimming	-	-	+	+
Loss of Reflex	-	-	-	+
Hyperventilation	-	-	+	+
Motionless State	-	-	-	-
Surfacing	-	-	+	+
Discoloration	-	-	-	-

3.4 Difference in the Acute Toxicity Assay

Tables 4 and 5 show the summary results of the One-way Analysis of Variance (ANOVA) and DMRT of the acute toxicity assay conducted to *O. niloticus* fishes within the 96 h exposure to leachate. Results revealed a high significant difference between treatment groups and 100 ppm as the most potent concentration.

Table 4. Results of the One-way Analysis of Variance (ANOVA) of the acute toxicity assay carried out to *O. niloticus* fishes within 96 h of exposure.

Sources of Variation	Sum of Squares	Df	Mean Square	F-ratio	p
Between Groups	133.375	3	44.458		
Within Groups	0.500	4	0.125	355.667	.000**
Total	133.875	7			

** $p < 0.01$

Table 5. Results of the Post-hoc Analysis using Duncan's Multiple Range Test carried out to *O. niloticus* fishes within 96 h of exposure.

Treatment (I)	Treatment (J)	Mean Difference	Sig.
Control a	10 ppm b	.00000	1.000 ^{ns}
	50 ppm b	-3.50000	.002**
	100 ppm c	-10.00000	.000**
10 ppm	50 ppm b	-3.50000	.002**
	100 ppm c	-10.00000	.000**
50 ppm	100 ppm c	-6.50000	.000**

** $p < 0.01$

Treatments with the same letter are not significantly different.

3.5 Median Lethal Concentration

Figure 1 illustrates the lethal concentration of the Bacolod City Sanitary Landfill leachate within the 96 h exposure using Finney's Probit Analysis at 57.688 ppm.

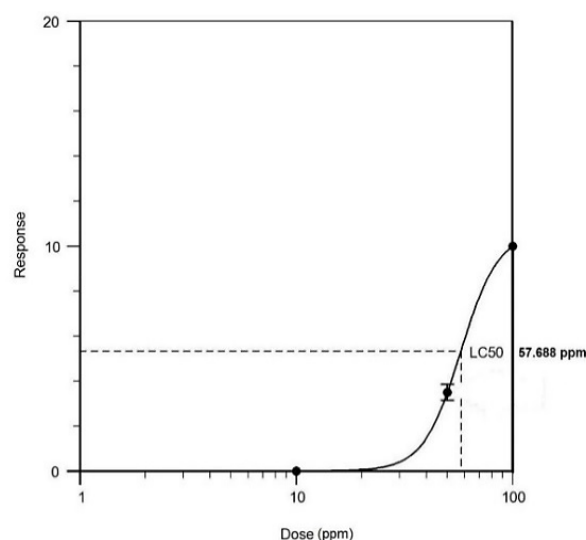


Fig. 1. Lethal concentration of leachate carried out to *O. niloticus* fishes within 96 h of exposure.

The results of LC50 are very significant to assess the concentration of toxics and pollutants present on the sanitary landfill. According to the Organization for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals, LC values denotes to the concentration of a chemical in water when testing for environmental studies. Also, the results of the LC50 will give a standard level of comparison between different samples leachate as to which leachate sample will be more toxic.

4 Conclusions

With the forgoing findings of the present research study, the following conclusions are drawn in light of the investigation:

1. Physicochemical analysis data of leachate samples collected formed the quality database for the study. Color, chemical oxygen demand (COD) and total dissolved solids (TDS) surpassed the standard limit established by DENR DAO 2016-08.
2. Toxic effects of heavy metals such as zinc and lead in leachate samples of the Bacolod City Sanitary Landfill have been demonstrated in the present study which exceeded the permissible standard limit set by DENR DAO 2016-08.
3. Significant behavioral and physiological changes on *O. niloticus* species demonstrated surfacing, erratic swimming, hyperventilation, and loss of reflex.
4. The median lethal concentration of 57.688 ppm or 0.057688 mL/L is a very toxic dose of leachate in which half of the population of the *O. niloticus* fishes was extirpated.
5. The increasing concentration of leachate was associated with the increasing mortality rate based on results obtained using a One-way ANOVA.
6. Among all the concentrations used in the research study, the most potent leachate concentration that can eradicate *O. niloticus* fishes when exposed was 100 ppm or 0.01 mL/L basing on the results obtained from Post-hoc Analysis using Duncan's Multiple Range Test (DMRT).

A coordinated working relationship between and among LGUs, NGOs and private sector must be established for the strict implementation of the Ecological Solid Waste Management Act of 2000 (R.A. 9003) and plans for developing city and municipal wastewater treatment plants.

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