Toxicity of Various Organic Substances in Wastewater and Evaluated on *Brachydanio rerio*

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Abstract. High content of pollutants can be harmful to the biota in salt water and fresh water. There is a change in the level of danger from substances that are not mixed to mixed. This study is important and necessary for the discharge of wastewater. The objective was to obtain a varied range of substances and determine the quality of wastewater discharging to fresh waters. COD analysis using gas chromatography. Toxicity test on *Brachydanio rerio* used Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Pow variability and BOD/COD ratio had a negative correlation of -0.486 (Pearson Sig 95%). Diazinon-formaldehyde-IPA showed the lowest LC-50 (46.69 mg/L) in *B. rerio*. There is compatibility between wastewater in the field and artificial wastewater in a mixture of organic acetic acid-IPA-formaldehyde in the biodegradable zone. The results of the BOD/COD ratio of the real material are similar to the reference material range in a mixture of organic matter acetic acid-IPA-formaldehyde in the biodegradable zone. It shows that acetic acid-IPA-formaldehyde can be used to compare the six types of real materials at any concentration.

Keywords: Toxicity; Organic Substances; Wastewater; *Brachydanio rerio*

1 Introduction

Wastewater with high pollutant content can harm the biota in it, both in water and fresh water. Hence, it is necessary to verify laboratory results with toxicity tests to determine the quality of disposal in water and fresh water. The LC50-96h toxicity test was carried out using three types of bioindicators, i.e., B. rerio due to its biological and reproductive properties (short generation interval and short spawning), which is suitable as test fish for toxicological research [1]. B. rerio is also used as a bioindicator in various types of processing [2].

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Analysis of BOD5, COD, and Pow was used to determine the long-range ratio of the six types of mixtures of organic substances as well as artificial wastewater compared to wastewater in the field. The BOD/COD ratio indicates the biodegradability of wastewater, where the higher the ratio, the higher the biodegradability [3]. Pow describes the hydrophobic and hydrophilic properties of aquatic organics [4]. Pow is directly proportional to the toxicity level of a substance. Inversely proportional to the ratio of BOD/COD, where the smaller the value of the ratio of BOD/COD, the more non-biodegradable the compound. Thus, the more non-biodegradable organic substances, the more they tend to be biota [1].

The organic substances used in this study were a mixture of glycerol, ethanol, isopropyl alcohol, diazinon, and five other organic substances, i.e., lactose, sucrose, formaldehyde, acetic acid, and oxalic acid. These organic substances are often used, and many are wasted in the environment. Sucrose in the manufacture of food products serves as a sweetener and preservative. Lactose is found in milk (35-45%). Formaldehyde is found in household cleaning materials, paper, shampoo, deodorant, toothpaste, lipstick, nail dye, and pesticides. Oxalic acid is used in textile manufacturing, metal surface processing, and leather tanning. Acetic acid is the most widely consumed and traded in industry and laboratories, known for its sour taste and aroma in food [5].

2 Method

2.1 Wastewater Characteristics

In this research, six types of wastewater with different characteristics were used. The wastewater was then compared with the artificial wastewater in this study. The characteristics of wastewater must be verified because it can determine the correct treatment method to avoid polluting the environment. The six types of wastewater are household, tofu industry, restaurants, shipyards, textiles, and leachate. Sampling was carried out in the effluent.

2.2 BOD_5, COD and Pow analysis

Variables tested include six mixtures of organic substances by analyzing BOD5, COD, and POW parameters. The BOD analysis was carried out using the Winkler method [6]. The COD analysis was performed with closed reflux spectrophotometry [7]. The next research stage was Pow analysis using gas chromatography [5].

2.3 Toxicity test LC_{50}-96h on *Brachydanio rerio*

ThddaToxicity testing methods for B. rerio were described by the USEPA in 2002 for Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. B. rerio used was about three months old with a length of 2 + 1 cm, a temperature of 26°C, and a water pH of 7.0 in a room maintained at a temperature of 24-26°C. The concentrations (10, 100, and 1000 mg/L) and control included ten fish and observed the number of deaths at 24, 48, 72, and 96 hours, then calculated the LC50 value using probit analysis. It comprises four series circuits. Each contained ten test animals with three different concentrations: 10 mg/L, 100 mg/L, 1000 mg/L, and control. The repetition was carried out three times [8].

3 Result and Discussion

3.1 Correlation between Pow variability and BOD/COD ratio

Based on the Pearson correlation analysis results, the significance value of the relationship between the BOD/COD and Pow ratios was obtained at 0.006, with N being the amount of data. Table 1 shows a correlation between the two because the significance value is <0.05. The BOD/COD ratio negatively correlates with Pow with a moderate degree of correlation. It can be observed from the Pearson correlation value obtained at -0.486. It demonstrates that the correlation contained in these factors has the opposite correlation. It means that if the BOD/COD ratio is low, the Pow value will be higher, while if the BOD/COD ratio value is high, the Pow value will be low.

Table 1. Pearson correlation (Sig-95%) between Pow variability and BOD/COD ratio

		Rasio BOD/COD	Pow
Rasio BOD/COD	Pearson Correlation	1	-486**
	Sig. (2-tailed)		0,006
	N	30	30
Pow	Pearson Correlation	-486**	1
	Sig. (2-tailed)	0,006	
	N	30	30

** Correlation is significant at the 0,01 level (2-tailed)

3.2 Comparison of Artificial Wastewater with Wastewater in the Field

Based on the BOD/COD ratio of wastewater in the field, the results are similar to the range of artificial wastewater in a mixture of acetic acid-IPA-formaldehyde organic substances in the biodegradable zone. It shows that a mixture of acetic acid-IPA-formaldehyde organic substances can be used to compare the six types of wastewater in the field at any concentration.

From the Pow results obtained, household and textile waste can be treated using biological treatment such as oxidation ditch, activated sludge, and aeration since the Pow value of the two types of wastewater was <1. Meanwhile, the other four types of wastewater had a Pow value > 1 (Table 2). Therefore, their treatment can use biological treatment with plants, such as phytoremediation, constructed wetlands, and aerobic biofilters, because biomass absorbs the waste from the processing.

For restaurant waste, phytoremediation can be used with aquatic plants, i.e., wlingen (Scripus grossus) and lotus (Nymphaea firecrest). Lotus could reduce up to 59.35% BOD, while wlingen could reduce up to 58.23% BOD [9]. As for phytoremediation, another plant effective for reducing high levels of BOD is the kiambang (Salvinia molesta). Kiambang effectively reduced BOD and COD concentrations by 17% and 83%, respectively [10].

Table 2. Test results of BOD/COD and Pow ratios of 6 types of wastewaters

No	Sample Code	COD (mg/L)	BOD_5 (mg/L)	BOD/COD Ratio	Pow
1	Household	200,00	108,00	0,540	0,36
2	Tofu Industry	7200,00	3946,00	0,548	1,43
3	Restaurant	16320,00	8696,00	0,532	2,65
4	Shipyard	2800,00	1270,00	0,453	1,12
5	Textile	900,00	446,00	0,464	0,98
6	Leachate	56400,00	30812,00	0,546	1,38

Tofu industrial waste can use phytoremediation with Azolla microphylla and water hyacinth plants. A. microphylla effectively reduced COD and BOD content by 96% [11]. Water hyacinth effectively reduced COD and BOD content by 96% [12]. As for phytoremediation, red spinach is a plant effective for reducing high levels of BOD, while kale can reduce high levels of COD [13].

The leachate can be performed using filtration and phytoremediation of artificial wetland systems using water bamboo plants (Equisetum hyemale). Filtration uses gravel, coconut husk, charcoal, sand, and zeolite stone. The study results showed a positive reaction, where DO increased by 7.8 ppm - 14.05 ppm [14]. In addition, leachate treatment with aquatic plants is effective on plants with long and fibrous roots. Eriocaulon sexangulare and Typha angustifolia effectively reduced BOD and COD, reaching >60% [15].

3.3 Toxicity test LC_{50}-96h on *Brachydanio rerio*

Based on the test results in Table 3, the smallest LC50-96h value in the diazinon-formaldehyde-IPA mixture was 46.69 mg/L. The lower the LC50 value, the more toxic a compound is. A substance is said to be very toxic if it has a small LC50 value and vice versa. The response of B. rerio to the mixed concentration of diazinon-formaldehyde-IPA showed the highest mortality.

Table 3. LC50-96h Value of Mixed Organic Substances on *B. rerio*

No	Mixed Organic Substances	LC50 Value (mg/L)	Probit Line Equation
1	Diazinon-formaldehyde-IPA	46,69	m=2,452x+0,907
2	Formaldehyde-ethanol-oxalic acid	59,40	m=2,511x+0,546
3	IPA-glycerol-lactose	140,78	m=1,804x+1,124
4	Acetic acid-IPA-formaldehyde	191,09	m=1,6x+1,35
5	Oxalic acid-formaldehyde-diazinon	52,18	m=2,563x+0,598
6	Sucrose-glycerol-acetic acid	205,09	m=1,715x+1,035

Note: m = mortality of the test organism and x = concentration of a mixture of organic substances.

The results of the LC50 toxicity test are acceptable if 90% of the control animals at the end of the observation were still alive. If survival is less than 90%, the test must be repeated [8]. In this study, the final result of the test organism in control at the end of the observation was 100%, so the results were acceptable. The relationship between mortality and pollutant concentration shows that the greater the increase in pollutant concentration, the greater the mortality. The smallest LC50 toxicity test result was found in the mixture of diazinon-formaldehyde-IPA. The equation for the probit line is m = 2.452x + 0.907, where m is the dependent variable indicating the mortality of the test animals for 96 hours. The value of a is the y-intercept of the value of 0.907, and b is the slope of the regression line, whose value is 2.452. Then x is the concentration of the mixture of organic substances. The positive regression coefficient (0.8862) indicates that the higher the concentration of a mixture of organic substances, the higher the mortality of the test animals, as in Fig. 1.

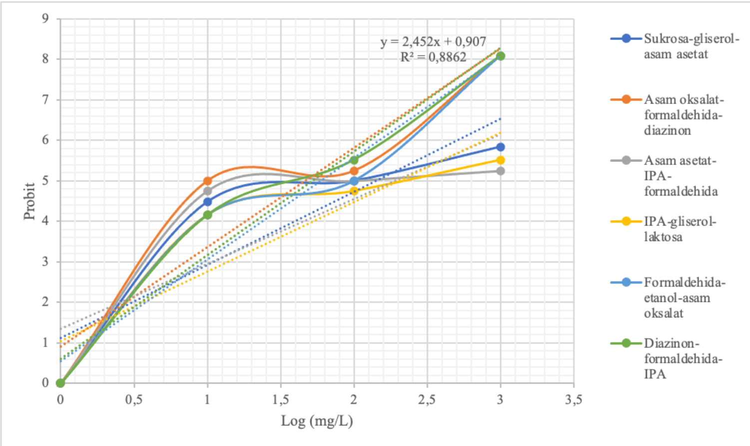

Fig. 1. Probit line equation of *B. rerio*

In the observation of B. rerio, physical symptoms were seen during initial exposure to waste. The test animals were hyperactive, and over time, there was no more movement. Observations on the toxicity test showed early symptoms due to exposure after 24 hours at all concentrations (Fig. 2a). After 24 hours of exposure, most of B. rerio tended to be hyperactive and then reversed before accumulating at the bottom and not actively moving until finally completely dead after 96 hours of exposure. Dead B. rerio will be white and sink (Fig. 2d).

Test animals exposed to toxic contaminants can be identified by hyperactive movements, floundering, paralyzing, and dying. Clinically, the poison-contaminated test animals showed symptoms of stress when compared to controls, characterized by less stable movements, and tended to be at the bottom. It is believed to be a way to minimize the biochemical processes in the poisoned body to allow lethal effects to occur slower [16].

According to [17], the death of test animals is not always caused by a single factor. It can also be caused by a synergistic phenomenon, i.e., combining two or more substances that strengthen the toxicity. The lethal effect of a pollutant on living things is the response that occurs when the pollutant interferes with cell or subcellular processes in living things to the extent that it causes direct death [18].

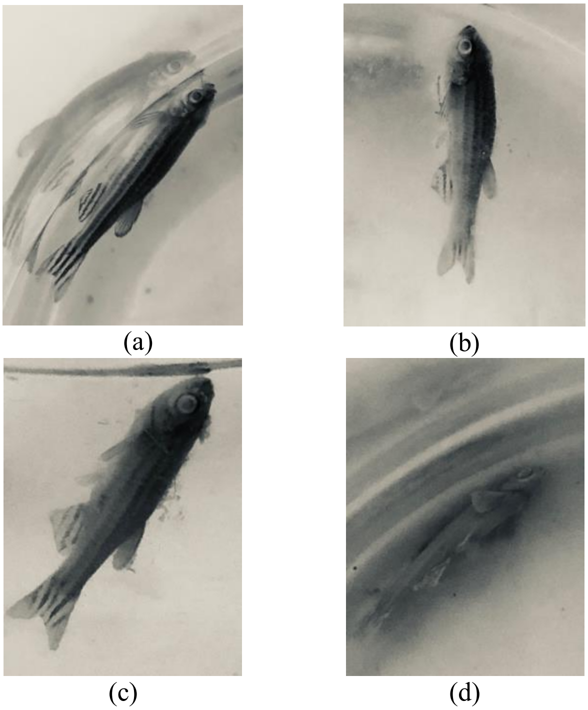

Fig. 2. Physical condition *B. rerio* (a) 24, (b) 36, (c) 72, and (d) 96 hours

The results of the LC50-96h test on B. rerio have verified the laboratory analysis carried out: BOD5, COD, and Pow. Diazinon-formaldehyde-IPA had the smallest LC50-96h. Hence, the response with the highest mortality was obtained. It aligns with the biodegradability of the mixture of organic substances in the non-biodegradable category. Meanwhile, sucrose-glycerol-acetic acid and IPA-glycerol-lactose had the largest LC50-96h values and were included in the biodegradable category in laboratory analysis. It also follows the biodegradability of the mixture of organic substances included in the biodegradable category.

4 Conclusion

The results of the LC50-96h test on B. rerio have verified the laboratory analysis carried out, i.e., BOD5, COD, and Pow. Diazinon-formaldehyde-IPA had the smallest LC50-96h. Therefore, the response with the highest mortality was obtained. It aligns with the biodegradability of the mixture of organic substances in the non-biodegradable category. Meanwhile, sucrose-glycerol-acetic acid and IPA-glycerol-lactose had the largest LC50-96h values and were included in the biodegradable category in laboratory analysis. It also follows the biodegradability of the mixture of organic substances included in the biodegradable category.

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