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Evaluation of the LC_{50} (Lethal Concentration) for LAS and Pb Administration

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A B S T R A C T

The surface water quality in Indonesia is deteriorating due to the accumulation of waste, particularly detergent waste from households and heavy metal waste from industries. This pollution source contributes to the loss of aquatic life. This study aims to determine the LC50 value for aquatic biota, specifically goldfish, and provide recommendations for relevant stakeholders. Acute toxicity tests were conducted over 96 hours using goldfish (Cyprinus carpio L) test subjects, with variations in Linear Alkylbenzene Sulfonate (LAS) and Lead (Pb) concentrations to ascertain the concentration causing 50% mortality, determined through a range-finding test. The toxicity test stage employs various concentrations of toxicants identified during the Range Finding Test stage, including: P0 (0 mg/L both LAS and Pb), P1 (0.05 mg/L LAS & Pb), P2 (0.1 mg/L LAS & Pb), P3 (0.15 mg/L LAS and Pb), P4 (0.2 mg/L LAS and Pb), P5 (0.25 mg/L LAS and Pb) and P6 (0.3 mg/L LAS and Pb). The LC50 value for LAS and Pb was established at 0.313 mg/L. This research can serve as an initial reference for biomonitoring detergent and heavy metal waste in aquatic environments. Effective effluent control is crucial for both industries and households to ensure compliance with government-mandated concentration standards for detergent and heavy metal effluents.

Keywords: Goldfish, LC_{50}, Lead (Pb), Linear Alkylbenzene Sulfonate (LAS)

INTRODUCTION

One of the freshwater sources in Indonesia is located in rivers and lakes, but its quality is declining due to the accumulation of pollutants, particularly detergent waste from industries and households. The term "detergent" refers to a broad range of cleaning substances used to clean dirt from numerous dirty items. The primary components of detergents are organic compounds known as surfactants, which are "surface active" in water. Synthetic surfactants are categorized into three main types: anionic, nonionic, and cationic. An example of anionic surfactant is LAS (*linear alkyl sulfonate*) (Sawyer, 2003). The use of detergents containing linear alkyl benzene sulfonate (LAS) and fluorescent whitening agents (FWAs) is rapidly rising in Asian countries, including Indonesia (Katam *et.al*, 2018). Previous studies have indicated that surfactant levels in the river water of South Sumatra Province exceeded the quality standards established by the government (Nurhaliza & Sunarti, 2020). Another study reported that anionic surfactant levels in the Barito River exceed the acceptable threshold. Despite this, the Barito River, located in Ulu Benteng Village RT.11, Barito Kuala Regency, is still used by the community for daily needs, including washing clothes and as a drinking water source (Fajriah *et.al*, 2020). Surfactants can lead to environmental pollution, especially in rivers. According to Badmus *et al.* (2021), high concentrations of surfactants and their degradation products disrupt microbial dynamics and crucial biogeochemical processes, adversely affect plant survival and their ecological niches, and impede human organic and systemic functions. Additionally, the presence of LAS in aquatic environments adversely affects fish, impacting their development from the early stages of life through to adulthood. Studies have shown that exposure to detergents significantly alters the histological structure of the liver and gills in *Oreochromis niloticus*. The mean lethal concentration (LC_{50}) for the household detergent over 96 hours was 10 mg/L. Furthermore, the relative percentage of detergent residues in fish muscles increased as the detergent concentrations rose (Gouda *et.al*, 2022).

In addition to organic pollutants like LAS that can contaminate water, there are also hazardous inorganic materials, such as heavy metals like lead, which pose significant risks to aquatic environments. Multiple studies have shown that fish can bioaccumulate trace elements, particularly heavy metals, in their tissues, and subsequently transfer these elements to humans through consumption. It has been proven that fish samples collected from dams in Osun State, Nigeria, contain concentrations of potentially toxic elements (PTEs) such as cadmium, chromium, nickel, and lead that exceeded the maximum permissible levels (Ishola *et.al*, 2023). Meanwhile in Indonesia, Safitri *et al*. (2022) reported significant accumulation of heavy metal lead in the meat, skin, liver, kidneys, and gills of baung fish (*Hemibagrus nemurus*) from the Musi River, South Sumatra. The concentrations in the liver, kidneys, and gills exceeded the quality standards set by the Indonesian Food and Drug Administration (BPOM) Regulation Number 5 of 2018.

Contamination of food sources by heavy metals is a significant environmental and health issue due to the adverse health effects associated with consuming food contaminated with heavy metals, particularly fish-derived products. Hence, conducting risk assessments is essential to anticipate potential risks and identify suitable solutions. The fish acute toxicity test is a widely used method in aquatic ecotoxicology to assess environmental risk. These tests aim to determine the concentration that would be lethal to 50% of the exposed animals (Burden *et.al*, 2020).

MATERIALS AND METHOD

Study Area

This experimental research was conducted at the Environmental Engineering Laboratory, Faculty of Life Science and Technology, UIN Sunan Ampel Surabaya, Indonesia.

Research Framework

This study utilized goldfish *(Cyprinus carpio L.)* obtained from Lamongan Regency. The selected fish had lengths ranging from 4 to 7 cm and were aged between 1 to 3 months. The fish sample is represented in Figure 1.

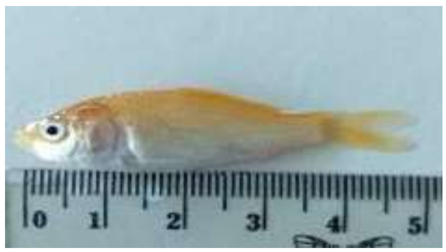

Figure 1. Sample of goldfish *(Cyprinus carpio L.)*

In this study, the goldfish *(Cyprinus carpio L.)* (biota) were subjected to a series of treatments, which included the following stages:

Acclimatization

The acclimatization stage, which lasts for 7 days, aims to eliminate stress in the test animals. During this period, the test biota was fed daily and provided with adequate aeration to ensure sufficient dissolved oxygen levels. Fish mortality was monitored daily, and any dead fish were promptly removed from the aquarium.

The Range Finding Test

The range finding test is conducted to identify the critical concentration range that results in approximately 50% mortality of the test biota. This serves as the basis for determining the lethal concentration used in subsequent experiments. Range Finding Test was conducted for 96 hours according to Table.1.

Table 1. LAS & Pb Concentration for Range Finding Test

Treatments	LAS (mg/L)	Pb (mg/L)
P_0	0	0
P_1	0.1	0.1
P_2	0.2	0.2
P_3	0.3	0.3
P_4	0.4	0.4
P_5	0.5	0.5
P_6	0.6	0.6

Toxicity Test

This stage involved exposing the fish to different concentrations of the toxic substances for 96 hours. Fish mortality was documented every 24 hours.

LC_{50} Determination

The final stage involved calculating the $LC5_0$ value using the probit regression method. The probit regression analysis was performed using SPSS software version 25.

RESULTS AND DISCUSSION

Acclimatization

The results of the goldfish *(Cyprinus carpio L.)* acclimatization stage are shown in Figure 2. Four fish deaths were recorded during the acclimatization stage. These deaths were attributed to the inability of the test animals to adapt to the new aquatic environment, leading to stress. However, considering that the number of deaths accounted for ⩽ 2% of the total 225 test fish used, they are considered insignificant.

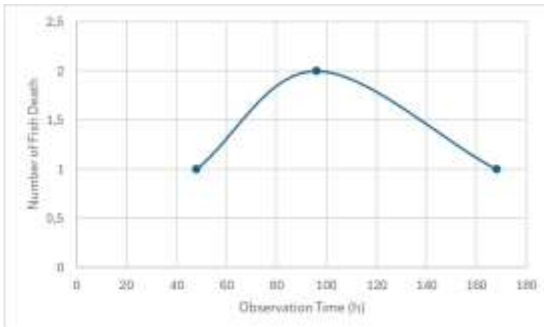

Figure 2. The results of the goldfish *(Cyprinus carpio L.)* acclimatization stage

Range Finding Test

The results of the range finding test Stage of goldfish *(Cyprinus carpio L.)* are presented in Figure 3. Based on the findings from the Range Finding Test, it's evident that goldfish *(Cyprinus carpio L.)* mortality reached 100% at a concentration of 0.6 mg/L for both LAS and Pb waste. Fish mortality reached 90% in the media containing 0.5 mg/L LAS and Pb, while other concentrations below 0.3 mg/L yielded the fish mortality of 50%. There was no fish mortality in media that did not contain both toxicants. These findings indicate that the rapid occurrence of fish mortality underscores the harmful effects of the waste on aquatic organisms, particularly with elevated levels of LAS and Pb exceeding quality standards. Exposure of goldfish *(Cyprinus carpio L.)* to higher concentrations of LAS and Pb waste led to increased mortality within a relatively short test duration of 96 hours.

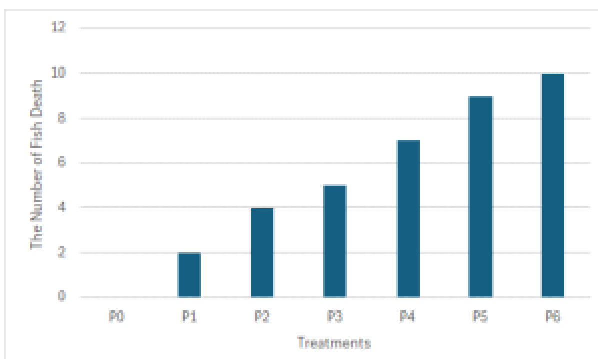

Figure 3. The results of the Range Finding Test Stage of goldfish *(Cyprinus carpio L.)*.

Toxicity Test

The toxicity test stage employs various concentrations of toxicants identified during the Range Finding Test stage. The LAS and Pb concentration for the toxicity test are presented in Table 2.

Table 2. LAS and Pb concentration for the toxicity test

Treatments	LAS (mg/L)	Pb (mg/L)
P_0	0	0
P_1	0.05	0.05

P_2	0.1	0.1
P_3	0.15	0.15
P_4	0.2	0.2
P_5	0.25	0.25
P_6	0.3	0.3

The toxicity test of goldfish *(Cyprinus carpio L.)* finding is presented in Figure 4.

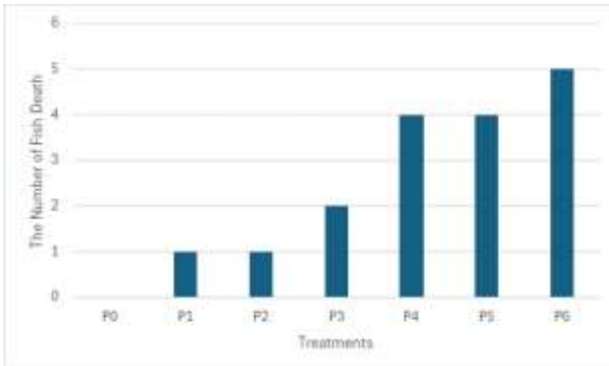

Figure 4. The toxicity test of goldfish *(Cyprinus carpio L.)* results

Based on the findings of the acute toxicity test, no goldfish died in the media that did not contain LAS and Pb, with concentrations of both toxicants being 0 mg/L. However, in media containing 0.05 mg/L and 0.1 mg/L of LAS and Pb, an average of 1 fish died. At a concentration of 0.15 mg/L of LAS and Pb, 2 fish died. This number increased to 4 fish at 0.2 mg/L and 0.25 mg/L toxicants concentration and reached 5 dead goldfish at 0.3 mg/L concentration. These mortality rates were recorded as averages over observation periods of 24 to 96 hours. These results indicate that higher concentrations of toxicants (LAS and Pb) correlate with increased fish mortality.

LC_{50} Determination

The results of the probit regression analysis for determining the LC_{50} are presented in Table 3. The probit analysis results indicate that the LC_{50} value of LAS and Pb for goldfish *(Cyprinus carpio L.)* is 0.313 mg/L.

Table 3. The probit regression analysis for determining the LC_{50}

The Probability		**95% of Confidence Limits for concentration**			**95% of Confidence Limits for log (concentration)[a]**		
		Estimate	**Lower Bound**	**Upper Bound**	**Estimate**	**Lower Bound**	**Upper Bound**
	,010	0,062	.	.	-1,205	.	.
	,020	0,075	.	.	-1,123	.	.
	,030	0,085	.	.	-1,071	.	.
	,040	0,093	.	.	-1,032	.	.
PROBIT	,050	0,100	.	.	-1,000	.	.
	,060	0,106	.	.	-,973	.	.
	,070	0,112	.	.	-,949	.	.
	,080	0,118	.	.	-,928	.	.
	,090	0,123	.	.	-,908	.	.

,100	0,129	.	.	-,891	.	.
,150	0,152	.	.	-,817	.	.
,200	0,175	.	.	-,758	.	.
,250	0,196	.	.	-,708	.	.
,300	0,217	.	.	-,663	.	.
,350	0,239	.	.	-,621	.	.
,400	0,262	.	.	-,581	.	.
,450	0,287	.	.	-,543	.	.
,500	0,313	.	.	-,505	.	.
,550	0,341	.	.	-,467	.	.
,600	0,373	.	.	-,429	.	.
,650	0,408	.	.	-,389	.	.
,700	0,449	.	.	-,347	.	.
,750	0,499	.	.	-,302	.	.
,800	0,560	.	.	-,252	.	.
,850	0,641	.	.	-,193	.	.
,900	0,759	.	.	-,120	.	.
,910	0,791	.	.	-,102	.	.
,920	0,827	.	.	-,082	.	.
,930	0,869	.	.	-,061	.	.
,940	0,918	.	.	-,037	.	.
,950	0,977	.	.	-,010	.	.
,960	1,051	.	.	,022	.	.
,970	1,150	.	.	,061	.	.
,980	1,296	.	.	,113	.	.
,990	1,566	.	.	,195	.	.

Discussion

Toxicity refers to the relative capacity of a substance to produce harmful effects in living organisms. This capacity is influenced by various factors, including the species, the dosage, and the route of exposure. Acute toxicity tests were conducted to determine the impact of LAS detergents and lead (Pb) waste at various concentrations on the mortality of test animals, specifically goldfish *(Cyprinus carpio L.)*. This study establishes a 96-hour Lethal Concentration 50 (LC_{50}-96h) value, which serves as a reference for initial biomonitoring of detergent and heavy metal waste in aquatic environments. The LC_{50} value of LAS and Pb for goldfish *(Cyprinus carpio L.)* is determined to be 0.313 mg/L. Previous researchers conducted acute toxicity tests on the effects of insecticide effluent on goldfish *(Cyprinus carpio L.)* and tilapia, finding LC50-96h values of 0.03 mg/L for goldfish *(Cyprinus carpio L.)* and 0.08 mg/L for tilapia. It was also noted that as the concentration of insecticide increased, the percentage of test fish mortality also increased. Conversely, when the concentration of insecticide was lower, the percentage of fish mortality decreased (Ihsan *et.al*, 2018). Other researchers reported that probit analysis of streptomycin and tetracycline antibiotics using goldfish *(Cyprinus carpio L.)* yielded an LC_{50} value of 2.0224 ppm over 96 hours. This indicates that a concentration of 2.0224 ppm can kill 50% of the test fish within 96 hours (Husein *et.al*, 2023).

Toxicity is a fundamental characteristic of various chemical substances, including acids and bases, heavy metals, chlorinated molecules, organic solvents, and protein toxins. Specific chemical structures are typically linked to particular modes of chemico-biological interactions and their corresponding adverse effects on living organisms (Schwenk & Burr, 2021). Fish mortality resulting from exposure to toxic substances like LAS and Pb is believed to happen when these substances enter through the gills, which serve as the primary site for gas exchange. The toxins then enter the bloodstream and disrupt the function of nerve-regulating enzymes, causing uncontrolled nerve activity. This ultimately leads to the death of the fish. Current study found that heavy metals like arsenic cause severe physiological damage and biochemical disorders in fish, including gill and liver poisoning, reduced fertility, tissue damage, lesions, and cell death. Arsenic also penetrates cells and generates reactive oxygen species, increasing stress levels and leading to elevated oxidative enzyme and cortisol levels in fish (Malik *et.al*, 2023). Gouda *et.al* (2021) also observed behavioral changes in Nile Tilapia exposed to detergent, noting that their opercular movements were faster compared to the control group. The fish frequently surfaced, showed signs of nervous control loss, and tried to jump out of the contaminated water. In the dead fish, the opercular region turned blackish, and there were hemorrhages on the lower lip, along the mid-ventral line behind the mouth, between the pectoral fins, and at the bases of the anal and pelvic fins. Similar to heavy metals, LAS (linear alkyl sulfonate) also has detrimental effects on fish morphology and physiology. LAS affects fish glucose levels, as reported by Rizkiya *et.al* (2023), who found that blood glucose levels in cere fish rise with increasing concentrations of LAS. The highest observed level was 95.6 mg/dl, which exceeds normal glucose levels in fish. Additionally, LAS surfactants can cause various types of damage to the gills and liver. Gills may suffer from edema, lamellar fusion, hyperplasia, lamellar epithelial swelling, and gill filament necrosis. In the liver, LAS can lead to blood vessel and sinusoid blockage, hyperplasia, sinusoid widening, fat accumulation, and hepatocyte necrosis.

CONCLUSION AND RECCOMENDATION

This study found that the LC_{50} value of LAS and Pb for goldfish (*Cyprinus carpio* L.) was 0.313 mg/L. These findings confirm that detergents and lead in aquatic environments have a severe impact on fish and pose a significant threat to their survival. This study can serve as a reference for the initial biomonitoring of detergent and heavy metal waste in aquatic environments. Effluent control is essential for both industries and households to ensure that detergent and heavy metal effluents adhere to government-set concentration standards.

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