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Acute Toxicity of Household-Scale Lebak Batik Industrial Wastewater on Common Carp

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ABSTRACT

The concentration of Lebak Batik industrial wastewater contains the pollutant element of lead metal that negatively impacts organisms' content in the water. This study was focused on the acute toxicity of household-scale Lebak Batik industrial wastewater on carp. The methods used were waste characteristics test, animal acclimatization, acute toxicity test for 96 hours with 0, 10, 20, 30, 40, and 50% treatments, measurement of environmental parameters, and data analysis using probit analysis. The results showed that the lethal time (LT_{50}) and lethal concentration (LC_{50}) for 96 hours of treatment were 1,8 days and 8,56%, respectively, with a Toxicity Unit Area (TUa) of 11,68. This proved that the wastewater from the Lebak batik industry had a major effect on causing acute toxicity.

Toksisitas Akut Limbah Cair Industri Batik Lebak Skala Rumah Tangga pada Ikan Mas

ABSTRAK: *Konsentrasi limbah cair industri batik mengandung unsur pencemar berupa kandungan logam yang berdampak negatif bagi organisme yang hidup di perairan. Untuk itu, penelitian ini berfokus pada toksisitas akut dari limbah cair industri Batik Lebak skala rumah tangga pada Ikan Mas. Metode yang digunakan adalah uji karakteristik limbah, aklimatisasi hewan uji, uji toksisitas akut selama 96 jam dengan perlakuan 0, 10, 20, 30, 40, dan 50%, pengukuran parameter lingkungan, dan analisis data menggunakan analisis probit. Hasil penelitian menunjukkan bahwa nilai lethal time (LT_{50}) dan lethal concentration (LD_{50}) selama 96 jam perlakuan masing-masing adalah 1,8 hari dan 8,56%, dengan Toxicity Unit Area (TUa) senilai 11,68. Hal ini membuktikan bahwa limbah cair industri batik Lebak berpengaruh besar menyebabkan toksisitas akut.*

INTRODUCTION

The development of the textile industry in Indonesia has increased significantly. The batik industry is included in the textile industry, which uses the most water in its production process. This liquid waste generally comes from dying and washing batik cloth (Kusumaningratri &

Damayanti, 2016). Many industrializations processes often cause negative impacts. The presence of industrial waste materials in both solid and liquid forms can affect the surrounding environment. The composition of nutrient components, organic matter, and biomass is very important for the community to maintain the stability of aquatic ecosystems. Still, the stability relationship will be broken soon when there is the input of toxic materials. The entry of pollutants will reduce the biological potential of these aquatic resources (Hastuti dkk., 2016).

Some batik industry businesses lack knowledge about batik wastewater treatment technology, so many still dispose of the waste directly into water bodies. The next result will cause river water pollution and reduce the survival of aquatic biota, including fish. Toxicity studies on fish are one of the most effective methods to determine the negative impact of contaminants in the aquatic environment. Common carp (*Cyprinus carpio*) is usually used as a bioindicator species because this fish is the most important teleost fish cultivated in the world for commercial purposes and is also an organism classified as resistant and easy to maintain (Saucedo-Vence dkk., 2017).

The background of this research is the absence of a batik waste processing process carried out by Lebak batik industry entrepreneurs on a household scale. Knowledge of wastewater treatment by both the owner and employees is very low and assumes that the waste produced by batik does not harm the environment. There is no monitoring from the relevant agencies regarding the impact of batik production waste disposal so that batik employees here can easily dispose of wastewater directly into the river.

A previous study about the toxicity test of batik waste on aquatic biota, especially fish, has been carried out in other locations. With a concentration of 0.73% of the batik industry, wastewater can cause the attachment of foreign substances found in the cross-section of the Nila Gift fish seed scales, where longer exposure can cause death (Andriani & Hartini, 2017). Other aquatic biota mortality, namely carp, also occurred after exposure to batik industry wastewater at a concentration of 6% for 24 hours (Pratiwi dkk., 2016; Safauldeen dkk., 2019; Tangahu dkk., 2019). Meanwhile, the mortality rate of sepat fish exposed to batik liquid waste in Bangkalan Regency, East Java, occurred at an LC_{50} value of 0.186%. (Juliardi dkk., 2020).

To reduce the toxic effects, study on the toxicity test of batik waste on aquatic biota after processing has also been carried out, such as using phytoremediation methods and biosorption using algae and fungi in the toxicity test of batik industrial wastewater through processing using phytoremediation using water hyacinth, at a concentration of 5% it still causes death in carp (Susilo dkk., 2021). Lethal toxicity from batik waste also occurred at a concentration of 52.876 ppm with waste treatment through biosorption using algae and fungi (Lestari dkk., 2017). However, there is no available information regarding the toxicity test of batik industrial wastewater, either without treatment or with processing in Lebak Regency.

The novelty of this research is more focused on the household-scale Lebak Batik industry. This research was expected to provide initial information about the quality of batik wastewater from the Lebak batik industry to be used as a reference for wastewater management based on government regulations, methods and costs in its implementation. In addition, this study also aims to determine the acute toxicity of household-scale Lebak Batik industrial wastewater on carp.

METHOD

This research was conducted in March-May 2021 at The Integrated Laboratory of Science, Pharmacy and Health Faculty, University of Mathla'ul Anwar Banten to test the acute toxicity of batik industrial wastewater on carp and Water Quality Laboratory of Fish and Environmental Disease, Serang, Banten.

The tools used for this research were a fish rearing tank, jerry can, aerator, pH meter, thermometer, dropper, beaker glass, and Erlenmeyer. The materials used for this research are carp, Batik industrial wastewater, and commercial fish feed.

This research was an experimental study conducted using a completely randomized design with 6 treatment groups, namely carp exposed to batik industrial wastewater with various concentrations of 0, 10, 20, 30, 40, and 50%. The research procedures included testing waste characteristics, acclimatization of test animals, acute toxicity test for 96 hours with 0, 10, 20, 30, 40, and 50% treatments, measurement of environmental parameters, and data analysis. The details of this research procedure were as follows:

Batik Lebak wastewater characteristic test

Samples of liquid waste obtained from the Lebak batik industry on a household scale were taken randomly. The liquid waste is the final result of batik processing before being discharged into the environment. Furthermore, the characteristics of the waste were tested, including levels of Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and Total Suspended Solid (TSS).

Test animal acclimatization

The test animals used in this study were goldfish aged ±30 days with a bodyweight of 8-12 grams. Fish obtained from aquaculture ponds in the Batubantar area, Cimanuk District, Pandeglang Regency, Banten were acclimatized for 7 days with maintenance using an oxygen aerator and given commercial fish feed as much as 2.5% of the fish body weight.

Acute toxicity test

After acclimatization, as many as 162 common carp were placed in a fish rearing tank that had been given batik industry wastewater with various concentrations of 0, 10, 20, 30, 40, and 50%. The test was carried out for 96 hours. During the test, the fish were given oxygen using an aerator and given commercial fish feed as much as 2.5% of the bodyweight of the fish.

Data Analysis

The wastewater characteristic test data were analyzed descriptively, while the acute toxicity results were statistically analyzed using probit analysis to obtain 50% lethal time (LT_{50}) and 50% lethal concentration (LC_{50}). The results of the LC_{50} were used to determine the Toxicity Unit Area (TUa) value where the LC_{50} is converted using the following equation:

$$\mathrm{TUa} = \frac{1}{\mathrm{LC}_{50}}\, x\ 100\%$$

The TUa value from the concentration of waste cause 50% of organisms to die at the end of the acute toxicity test and provide a classification of the toxic class of waste.

RESULTS AND DISCUSSION

The results of testing the characteristics of the wastewater were carried out at the Water Quality Laboratory of Fish and Environmental Disease, Serang, Banten (Table 1). The results were then adjusted to the wastewater quality standards for textile businesses following Minister of Environmental Regulation Number 5 of 2014.

Table 1. Test Results of Liquid Waste Characteristics of the Lebak Batik Industry Household Scale

Parameter	Value (mg/L)	Quality Standard (mg/L)
BOD	2,6	60
COD	589	150
TSS	34,5	50

If discharged into the aquatic environment, parameters that pass these quality standards can result in negative effects on aquatic organisms (Bilotta & Brazier, 2008; Christin dkk., 2015). Based on Table 1, it can be seen that the BOD and TSS values did not exceed the quality standard, but the COD value was above the quality standard. This is different from the characteristic test of the batik industry wastewater in Yogyakarta, where BOD, COD and TSS values were obtained that exceed the quality standard (Joko dkk., 2016; Rahmadyanti & Audina, 2020). Even though this waste has gone through biological and physicochemical treatment processes (Indrayani & Triwiswara, 2018), another study that tested the quality of batik wastewater in Sidoarjo (Tangahu dkk., 2019) also produced COD and TSS values above the maximum limit for textile industrial wastewater (Andriani & Hartini, 2017). The high levels of COD in this study indicate that there were high concentrations of organic and inorganic materials that can harm the aquatic environment (Devi dkk., 2017).

The data obtained from the acute toxicity test of the Lebak Batik industrial wastewater on a household scale were the LT_{50}, LC_{50} and the TUa values. The LT_{50} value was obtained from carp mortality per day in all treatments (Table 2).

Table 2. Acute Toxicity Test Results of Lebak Batik Industry Liquid Waste Household Scale per Day

Days to-	Average Number of Dead Fish (fish)	Average Number of Test Fish (fish)	LT_{50} (days)
1	6	54	
2	40	54	1,8
3	42	54	
4	43	54	

The acute toxicity test results of the Lebak batik industrial wastewater on a household scale, when viewed per day, showed an increase in the number of fish that died (Table 2). The average fish mortality of 50% was represented in the LT_{50} value of 1.8 days. This indicates that the toxicant that entered the fish's body had affected the physiological processes in the fish's body on that day. It interfered with the survival of the fish (Efriadi dkk., 2018). The longer the fish was exposed to the toxicant, the higher the mortality rate. This is in line with the research results by (Lestari dkk. 2017; Little & Finger, 1990). Toxicity factors in batik wastewater results caused the experimented animal mortality. The fish mortality rate was getting higher from 0 to 100% concentration. Based on that observation finds, 50% death of the experimented animals in 4,2% and 5,6% scale concentration of batik wastewater. Even though, batik wastewater complexity makes it uneasy to determine kinds of death matter of the experimented animals. Other research found that some heavy metals from wastewater caused fish death. The presence of chromium in the fish body will cause cell rupture and interact with proteins and semi-permeable membranes. This condition can cause fish to experience physiological stress, affect health, and cause death (Arfiati dkk., 2019; Davis dkk., 2001; Schreck & Tort, 2016).

Table 3 and Figure 1 showed that the toxicity test of the Lebak batik industrial wastewater on a household scale harmed the survival of carp. 0% concentration no dead fish. However, at a 10-50% concentration, many fish died above 50%, even at a concentration of 20-50%, resulting in 100% mortality. The LC_{50} value of 8.56% indicated this. This means that it can give a lethal effect to 50% of the individuals tested at this concentration. Another study that tested the toxicity of batik industrial wastewater on tilapia stated that at a concentration of 1%, it caused 9.6% of fish mortality with an LC_{50} value of 96 hours at an interval of 0.625-0.75% (Andriani & Hartini, 2017). Susilo dkk., (2021) said in his publication that fish mortality is characterized by changes in behavior such as changes in movement activity, body balance, ramjet ventilation, mucus production, changes in morphological color.

Table 3. Results of Toxicity Test of Lebak Batik Industry Liquid Waste Household Scale with Different Concentrations

Concentrations (%)	Average Number of Dead Fish (fish)	Average Number of Test Fish (fish)	Fish Mortality Percentage (%)	LC_{50} (%)
0	0	9	0	
10	7	9	77	
20	9	9	100	8,56
30	9	9	100	
40	9	9	100	
50	9	9	100	

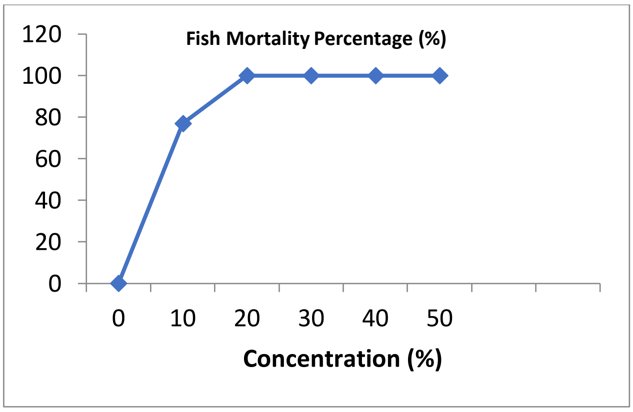

Figure 1. Fish Mortality Percentage on Different Concentrations of Lebak Batik Industry Liquid Waste Household Scale

Liquid waste toxicity tests can be classified into several levels. Level I is a category that does not cause acute toxicity with a value of TUa < 0.4, level II is a small category that causes acute toxicity with a value of 0.4 < TUa < 1, level III is a category that causes acute toxicity with a value of 1 TUa < 10, a level of IV is a large category causing acute toxicity with a value of 10 TUa < 100, and level V is a category that causes acute toxicity with a value of a 100 (Persoone dkk., 2003; Roopadevi & Somashekar, 2012). Based on the study results, the TUa value generated from the household scale Lebak Batik waste toxicity test for carp had a value of 11.68. This value was included in the classification of acute toxicity level IV, which was large, causing acute toxicity.

The environmental parameters measured during the study, namely pH and temperature (Table 4), showed that the pH and temperature were stable every day, ranging from 7-8 for pH and 27.6-29^{o}C. Based on the table, the 2nd day to the 4th day at a 20-50% concentration did not measure the temperature and pH because the fish died 100%. Based on previous research, a temperature of 20-28^{o}C had a good survival rate of common carp that was 100%. Still, the best growth was at a temperature of 28^{o}C with an increase in absolute length and absolute weight of 2.59 cm and 4.38 g, respectively (Gamperl & Syme, 2021; Ridwantara dkk., 2019). At the same time, the pH of the results of this study can be said to be good. Measurement of pH in common carp exposed to batik liquid waste from biosorption ranged from 6.0 to 7.0 (Sani dkk., 2020). Several factors that affect the high and low pH values are the decomposition process of organic matter in the water, the levels of gases, water temperature, and sunlight. The negative effects caused when there is a change in the pH of the water is that fish are susceptible to disease, fish metabolism is disturbed, fish growth is not well developed. The pH of the water that is good for fish life is between 6-9. If it exceeds 9, then the water is not suitable for the fish's living environment (Juliardi dkk., 2020; Kasumyan, 2019).

Table 4. Measurement results of environmental parameters (pH and temperature) during acute toxicity test treatment

Days to-	Treatment Group	Average pH value	Average Temperature Value (^{o}C)
1	0%	7,2	28,5
	10%	7,2	28,7
	20%	7,4	28,8
	30%	7,8	29,0
	40%	7,9	28,8
	50%	8,0	28,8

Days to-	Treatment Group	Average pH value	Average Temperature Value (^oC)
2	0%	7	27,8
	10%	7	28,1
	20%	-	-
	30%	-	-
	40%	-	-
	50%	-	-
3	0%	7	28,2
	10%	7	28,6
	20%	-	-
	30%	-	-
	40%	-	-
	50%	-	-
4	0%	7,3	27,6
	10%	7,4	27,8
	20%	-	-
	30%	-	-
	40%	-	-
	50%	-	-

The acute toxicity of wastewater from the Lebak batik industry to common carp was thought to be due to toxicants in the waste that cause physiological and anatomical damage to fish and cause death. The higher the concentration of exposure to batik waste as a medium for rearing carp, the darker the anatomical picture of the scales are. This is because the pigment cells in the carp's body are damaged. Dead fish will secrete mucus on the branchia and body surface. This mucus secretion is thought to be caused by batik dye waste in contact with the fish's body (Susilo dkk., 2021). The research results on the exposure of Sokaraja batik liquid waste and waste treatment, namely by biosorption, had an impact on anatomical damage to the kidneys. The microanatomical picture of the carp kidney indicates hypertrophy and necrosis of the tubules and necrosis of the glomerulus (Sani dkk., 2020; Tokarz & Wolf, 2022). In addition, the biosorption batik liquid waste also affects the physiological function of carp blood (Dietrich dkk., 2014), namely increasing the hematocrit value (Hidayati dkk., 2019; Moreno dkk., 2000; Rahmaniah dkk., 2019) leukocyte differential (Agustiana dkk., 2020; Dot dkk., 1992; Tang dkk., 2012).

CONCLUSIONS AND SUGGESTIONS

Based on the research and discussion results, it can be concluded that the household-scale Lebak batik industrial wastewater has a major effect on causing acute toxicity with a TUa value of 11.68. The acute toxicity test resulted in an LT_{50} of 1.8 days and an LC_{50} of 8.56%. The high acute toxicity was indicated because the COD level in the household-scale Lebak batik industrial wastewater had a value above the quality standard.

Suggestions that can be put forward for this research are that a waste treatment process is needed before being discharged into the environment, and it is necessary to socialize it to business actors so that the waste is not directly discharged into water bodies so as not to endanger the river ecosystem.

REFERENCES

Agustiana, H., Lestari, S., & Wibowo, E. S. (2020). Toksisitas Subletal Limbah Cair Batik hasil Biosorpsi terhadap Ikan Mas (Cyprinus carpio L .) ditinjau dari Differensial Leukosit. *Bioeksakta : Jurnal Ilmiah Biologi Unsoed*, *2*(2), 248–254.

Andriani, R., & Hartini, H. (2017). Toksisitas Limbah Cair Industri Batik Terhadap Morfologi Sisik Ikan Nila Gift (Oreochomis nilotocus). *Jurnal SainHealth*, *1*(2), 83–91. https://doi.org/10.51804/jsh.v1i2.108.83-91

Arfiati, D., Zakiyah, U., Nabilah, I. S., Khoiriyah, N., Jayanti, A. S., & Kharismayanti, H. F. (2019). Perbandingan LC50 – 96 jam terhadap mortalitas benih ikan mas, Cyprinus carpio Linnaeus 1758 pada limbah penyamakan kulit dan insektisida piretroid. *Jurnal Iktiologi Indonesia*, *18*(2), 103. https://doi.org/10.32491/jii.v18i2.430

Bilotta, G. S., & Brazier, R. E. (2008). Understanding the influence of suspended solids on water quality and aquatic biota. *Water Research*, *42*(12), 2849–2861.

Christin, F., Elystia, S., & Yenie, E. (2015). Uji toksisitas akut limbah cair tahu terhadap Daphnia magna dengan metode renewal test. *JOM FTEKNIK*, *2*(2), 1–9.

Davis, M. W., Olla, B. L., & Schreck, C. B. (2001). Stress-induced by hooking, net towing, elevated seawater temperature and air in sablefish: Lack of concordance between mortality and physiological measures of stress. *Journal of Fish Biology*, *58*(1), 1–15.

Devi, P. A., Padmavathy, P., Aanand, S., & Aruljothi, K. (2017). Review on water quality parameters in freshwater cage fish culture. *International Journal of Applied Research*, *3*(5), 114–120.

Dietrich, M. A., Arnold, G. J., Nynca, J., Fröhlich, T., Otte, K., & Ciereszko, A. (2014). Characterization of carp seminal plasma proteome in relation to blood plasma. *Journal of proteomics*, *98*, 218–232.

Dot, D., Miro, J., & Fuentes-Arderiu, X. (1992). Biological variation of the leukocyte differential count quantities. *Scandinavian journal of clinical and laboratory investigation*, *52*(7), 607–611.

Efriadi, H., Mutiara, D., & Emilia, I. (2018). Uji Toksisitas Akar Tuba (Derris eliptica) terhadap Mortalitas Benih Ikan Nila (Oreochromis sp). *Sainmatika: Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam*, *15*(1), 56. https://doi.org/10.31851/sainmatika.v15i1.1783

Gamperl, A. K., & Syme, D. A. (2021). Temperature effects on the contractile performance and efficiency of oxidative muscle from a eury-vs. Stenothermal salmonid. *Journal of Experimental Biology*.

Hastuti, B., Masykur, A., & Hadi, S. (2016). Modification of chitosan by swelling and crosslinking using epichlorohydrin as heavy metal Cr (VI) adsorbent in batik industry wastes. *IOP Conference Series: Materials Science and Engineering*, *107*(1). https://doi.org/10.1088/1757-899X/107/1/012020

Hidayati, N., Lestari, S., & Wibowo, E. S. (2019). Toksisitas Subletal Limbah Cair Batik Hasil Biosorpsi terhadap Hematokrit Ikan Mas (Cyprinus carpio). *Bioeksakta : Jurnal Ilmiah Biologi Unsoed*, *1*(2), 44–47.

Indrayani, L., & Triwiswara, M. (2018). Efektivitas pengolahan limbah cair industri batik dengan teknologi lahan basah buatan. *Dinamika Kerajinan dan Batik*, *35*(1), 53–66.

Joko, T., Putri, D. A. C., & Dangiran, H. L. (2016). Chitosan on reducing chemical oxygen demands in laundry wastewater. *International Journal of Sciences*, *30*(1), 104–111.

Juliardi, N. R., Andini, N., & AS, Y. (2020). Analisis Toksisitas Limbah Cair Batik Tulis dan Bioconcentration Factor Ikan Sepat (Trichogaster tricopterus). *Jurnal ENVIROTEK*, *12*(1), 19–26.

Kasumyan, A. O. (2019). The taste system in fishes and the effects of environmental variables. *Journal of fish biology*, *95*(1), 155–178.

Kusumaningratri, T. A., & Damayanti, A. (2016). *Impact of Competition Membran Chitosan and Zeolite*

Composite for Batik Colouring Wastewater Treatment. 205–213.

Lestari, S., Sudarmadji, Tandjung, S. D., & Santosa, S. J. (2017). Lethal toxicity of Batik wastewater bio-sorption results in Tilapia (Oreochromis niloticus). *Advanced Science Letters*, *23*(3), 2611–2613. https://doi.org/10.1166/asl.2017.8744

Little, E. E., & Finger, S. E. (1990). Swimming behavior as an indicator of sublethal toxicity in fish. *Environmental Toxicology and Chemistry: An International Journal*, *9*(1), 13–19.

Moreno, F., Sanz-Guajardo, D., Lopez-Gomez, J. M., Jofre, R., & Valderrabano, F. (2000). Increasing the hematocrit has a beneficial effect on quality of life and is safe in selected hemodialysis patients. *Journal of the American Society of Nephrology*, *11*(2), 335–342.

Persoone, G., Marsalek, B., Blinova, I., Törökne, A., Zarina, D., Manusadzianas, L., Nalecz-Jawecki, G., Tofan, L., Stepanova, N., Tothova, L., & Kolar, B. (2003). *A practical and user‐friendly toxicity classification system with microbiotests for natural waters and wastewaters* (hlm. 395–402).

Pratiwi, Y., Hastutiningrum, S., & Suyadi, D. K. (2016). Uji toksisitas limbah cair batik sebelum dan sesudah diolah dengan tawas dan super flok terhadap bioindikator (Cyprinus carpio L.). *Prosiding Seminar Nasional Aplikasi Sains & Teknologi (SNAST), November*, 571–579.

Rahmadyanti, E., & Audina, O. (2020). The Performance of Hybrid Constructed Wetland System for Treating the Batik Wastewater. *Journal of Ecological Engineering*, *21*(3).

Rahmaniah, G., Mahdi, C., & Safitri, A. (2019). Biosorption of Synthetic Dye from Batik Wastewater Using Trichoderma viride Immobilized on Ca-Alginate. *Journal of Physics: Conference Series*, *1374*(1), 012007.

Ridwantara, D., Buwono, I. D., Handaka, A. A., Lili, W., & Bangkit, I. (2019). Uji kelangsungan hidup dan pertumbuhan benih ikan mas (Cyprinus carpio) pada rentang suhu yang berbeda. *Jurnal Perikanan dan Kelautan*, *10*(1), 46–54.

Roopadevi, H., & Somashekar, R. K. (2012). Assessment of the toxicity of wastewater from a textile industry to Cyprinus carpio. *Journal of Environmental Biology*, *33*(2), 167.

Safauldeen, S. H., Abu Hasan, H., & Abdullah, S. R. S. (2019). Phytoremediation efficiency of water hyacinth for batik textile effluent treatment. *Journal of Ecological Engineering*, *20*(9).

Sani, K. I., Lestari, S., & Atang. (2020). Gambaran Histopatologis Ginjal Ikan Mas (Cyprinus carpio) yang terpapar Limbah Cair Batik Hasil Biosorpsi. *Bioeksakta : Jurnal Ilmiah Biologi UnsoedJurnal Ilmiah Biologi Unsoed*, *2*(1), 7–10.

Saucedo-Vence, K., Elizalde-Velázquez, A., Dublán-García, O., Galar-Martínez, M., Islas-Flores, H., SanJuan-Reyes, N., García-Medina, S., Hernández-Navarro, M. D., & Gómez-Oliván, L. M. (2017). Toxicological hazard induced by sucralose to environmentally relevant concentrations in common carp (Cyprinus carpio). *Science of the Total Environment*, *575*, 347–357. https://doi.org/10.1016/j.scitotenv.2016.09.230

Schreck, C. B., & Tort, L. (2016). The concept of stress in fish. Dalam *Fish physiology* (Vol. 35, hlm. 1–34). Elsevier.

Susilo, H., Oktavia, S., & Roudatussa'adah. (2021). Phytoremediation of batik industry wastewater using water hyacinth plant as a medium for maintaining Cyprinus Carpio L . *Biological Environment and Pollution*, *1*(1), 11–18.

Tang, H., Jing, J., Bo, D., & Xu, D. (2012). Biological variations of leukocyte numerical and morphologic parameters determined by UniCel DxH 800 hematology analyzer. *Archives of pathology & laboratory medicine*, *136*(11), 1392–1396.

Tangahu, B. V., Ningsih, D. A., Kurniawan, S. B., & Imron, M. F. (2019). Study of BOD and COD removal in batik wastewater using Scirpus grossus and Iris pseudacorus with intermittent exposure system. *Journal of Ecological Engineering*, *20*(5).

Tokarz, D. A., & Wolf, J. C. (2022). Animal Models in Toxicologic Research: Nonmammalian. Dalam *Haschek and Rousseaux's Handbook of Toxicologic Pathology* (hlm. 811–857). Elsevier.