

Heavy Metal Toxicity of Pb in the Organs of Nile Tilapia (*Oreochromis Niloticus***) in the Watershed Due to Hospital Waste**

Nindha Ayu Berlianti1* , Yuda Cahyoargo Hariadi2, Arry Yuariatun Nurhayati³ Wenny Maulina4, Firda Fadri⁵

1,2,3,4,5 Fisika, Universitas Jember, Jember, Indonesia ⁶ Matematika, Universitas Jember, Jember, Indonesia

A R T I C L E I N F O

A B S T R A K

Article history: Received July 28, 2024 Accepted October 13, 2024 Available online October 25, 2024

Kata Kunci: Logam Berat, Limbah Rumah Sakit, Ikan Nila, AAS

Keywords: Heavy Metals, Hospital Waste, Tilapia, AAS

This is an open access article under th[e CC](https://creativecommons.org/licenses/by-sa/4.0/) [BY-SA](https://creativecommons.org/licenses/by-sa/4.0/) license.

Copyright © 2024 by Author. Published by Universitas Pendidikan Ganesha.

Tingkat toksisitas logam berat yang terakumulasi di perairan dan sedimen akibat buangan limbah rumah sakit tidak hanya memberikan dampak pada lingkungan, tetapi juga mengancam kesehatan manusia apabila terakumulasi dalam tubuh dalam jangka panjang, yang berpotensi menyebabkan penyakit degeneratif seperti kanker. Alasan ini menjadi urgensi untuk melakukan penelitian dengan pendekatan eksperimen laboratorium guna mengidentifikasi sebaran logam berat di lingkungan perairan akibat limbah rumah sakit menggunakan bioindikator ikan nila (Oreochromis Niloticus), yang memiliki kemampuan untuk mengabsorpsi logam melalui jaringan tubuh. Subjek penelitian terdiri dari ikan nila sebanyak 35 ekor dengan panjang tubuh 8–12 cm dan berat 12–15 gram, serta sampel limbah rumah sakit yang diambil dari tiga stasiun saluran pembuangan. Metode pengumpulan data meliputi preparasi sampel limbah dan ikan nila, perlakuan dengan variasi konsentrasi limbah timbal nitrat (Pb(NO3)2) serta lama pemeliharaan selama 7, 14, 21, dan 28 hari. Pengukuran kandungan logam berat Pb dalam organ insang dan daging ikan nila dilakukan menggunakan Atomic Absorption Spectroscopy (AAS). Data hasil analisis berupa konsentrasi logam berat diplotkan pada grafik hubungan antara variasi konsentrasi dan lama pemeliharaan, serta diuji menggunakan analisis statistik One-Way ANOVA dengan uji nonparametrik Tukey. Hasil analisis menunjukkan konsentrasi logam berat Pb tertinggi ditemukan pada organ insang ikan nila pada hari ke-28 sebesar 1.57 ppm, sementara pada daging ikan nila sebesar 0.25 ppm pada hari ke-21. Berdasarkan standar BPOM, kandungan Pb pada daging ikan nila masih berada dalam batas toleransi aman untuk dikonsumsi (≤0.3 ppm), namun kandungan Pb pada organ insang telah melebihi batas toleransi. Penelitian ini memberikan bukti ilmiah mengenai risiko akumulasi logam berat pada biota air akibat limbah rumah sakit dan pentingnya pengelolaan limbah yang lebih baik untuk menjaga kesehatan masyarakat dan lingkungan.

A B S T R A C T

The toxicity levels of heavy metals accumulated in water and sediment due to hospital waste discharge not only impact the environment but also pose a significant threat to human health. Long-term accumulation of these metals in the body may lead to degenerative diseases such as cancer. This concern highlights the urgency of conducting laboratory-based experimental research to identify the distribution of heavy metals in aquatic environments caused by hospital waste. The study employs Nile tilapia (*Oreochromis Niloticus*) as a bioindicator, a species capable of absorbing metals through its tissues. The research subjects consisted of 35 Nile tilapia with a body length of 8–12 cm and a weight of 12–15 grams, alongside hospital waste samples collected from three discharge points. Data collection methods included sample preparation of both hospital waste and Nile tilapia, treatment with varying concentrations of lead nitrate (Pb(NO3)2), and maintenance durations of 7, 14, 21, and 28 days. The concentration of Pb in the gills and muscle tissue of the fish was measured using Atomic Absorption Spectroscopy (AAS). The analytical data, represented as heavy metal concentrations, were plotted on a graph showing the relationship between concentration variations and maintenance durations and analyzed using One-Way ANOVA with nonparametric Tukey's test. The analysis revealed that the highest Pb concentration was observed in the gills of Nile tilapia on day 28 at 1.57 ppm, while the Pb concentration in muscle tissue reached 0.25 ppm on day 21. According to BPOM standards, Pb levels in Nile tilapia muscle tissue remain within the safe consumption threshold $(≤0.3 ppm)$, whereas Pb levels in the gills exceeded the tolerance limit. This study provides scientific evidence on the risks of heavy metal accumulation in aquatic biota due to hospital waste and underscores the importance of improved waste management practices to safeguard public health and the environment.

1. INTRODUCTION

Hospital activities generate various types of waste containing pathogenic microorganisms that are both infectious and chemical in nature. Hospital waste falls into the category of hazardous and toxic waste (B3). If its management is not optimal and it is often disposed of carelessly into nearby river streams, it can lead to the spread of infectious diseases among the communities living around the watershed. These watersheds are drained through small rivers into river estuaries (Ermawati & Hartanto, 2017; Xu et al., 2022) . The accumulation and mixing of these wastes lead to a decline in river water quality (Khalil et al., 2018; Mester et al., 2022; Rahayu et al., 2018) . One of the chemical compounds present in hospital waste is heavy metals. The characteristics of heavy metals are highly dangerous and can lead to pollution in aquatic environments. High levels of heavy metal content can cause poisoning and damage to aquatic organisms. In previous studies, the identification of heavy metal content in aquatic environments caused by industrial and hospital waste was conducted, highlighting its contribution to the degradation of aquatic ecosystems. Heavy metal contamination in water sources is a major global environmental issue, posing threats to aquatic ecosystems and human health (Hama Aziz et al., 2023; Kirana et al., 2022). This study offers a novel approach by examining the accumulation levels of heavy metals, specifically lead (Pb), in Nile tilapia (Oreochromis niloticus) as a bioindicator of water pollution from hospital waste. Unlike previous research that primarily focused on direct analysis of heavy metal concentrations in water, this study provides deeper insights into heavy metal accumulation in specific fish organs, such as gills and muscle tissue, which are particularly relevant to human health and food safety. Using Atomic Absorption Spectroscopy (AAS), this research delivers more precise data in identifying bioaccumulation at the micro-level.

The urgency of this study stems from the increasing potential for pollution from hospital waste, which is often overlooked yet poses a significant threat to aquatic ecosystems and public health, especially given that water and aquatic biota in rivers are utilized for domestic needs, irrigation, and as a food source. Therefore, further identification of heavy metal accumulation in fish organs is crucial, forming the foundation for this study. It is hoped that the findings will provide scientific contributions to waste management efforts and serve as a reference for future research aimed at preserving aquatic ecosystems and protecting public health. The heavy metal content polluting the water bodies will naturally settle in the sediments and accumulate in aquatic organisms through bioaccumulation processes, Heavy metals tend to bind easily with organic compounds and settle at the bottom of the water, integrating with the sediment, which poses a potential risk of death if the absorbed content exceeds the permissible threshold limit x which is a maximum of 0.5 mg/kg allowed in fish (Azaman et al., 2015; Santi & Arsyad, 2021; Zuhairah et al., 2020). Moreover, heavy metals that accumulate in aquatic organisms can enter through the gills and flesh, ultimately reaching humans. If fish contaminated with heavy metals are consumed continuously over a long period, it can adversely affect human health (Noor, 2020; Pratiwi et al., 2019). Aquatic organisms, including Nile tilapia, are highly sensitive to heavy metal pollutants in affected water bodies, and have the potential to serve as bioindicators of water pollution (Alam et al., 2019; Azizah & Maslahat, 2021; Guevarra et al., 2020). Additionally, Nile tilapia are omnivorous aquatic animals increasing the potential for heavy metal accumulation in their tissues compared to other fish species (Berlianti et al., 2014; Busira et al., 2020). The quantity of metals accumulated in fish bodies depends on the type of compound and the concentration of pollutants in the water.

The watershed, which serves as the outlet for waste discharge, is extensively used as a water source for reservoirs, rice fields, and livestock ponds. This indicates that the watershed is widely utilized by the local community for daily activities as well as for their livelihoods. If humans consume Nile tilapia contaminated with heavy metals, it could trigger various health issues and diseases. Heavy metals in small amounts are necessary to maintain bodily balance, but in large quantities, they are harmful (toxic). The toxicity produced can reduce activity levels and impair nerve function, blood composition, liver, kidneys, and other vital organs. Over time, exposure can lead to physical degeneration, strength, and neurological damage, and gradually develop into diseases such as Parkinson's disease, Alzheimer's infection, multiple sclerosis, muscular dystrophy, and can also cause tumors (Shah, 2020; Yuniarti et al., 2023). This research aims to identify the heavy metal content in the watershed exposed to hospital waste, as the watershed in this region is used by the community for daily activities ranging from domestic tasks to livestock and agricultural irrigation systems. In the long term, preventive measures can be taken by conducting laboratory-scale tests to create variations in waste concentrations based on the reference data obtained from three station points in the watershed closest to the hospital liquid waste disposal site, located in a residential area.

2. METHOD

This study employs a laboratory experimental design with a quantitative approach to identify the accumulation of Pb heavy metal in the organs of Nile tilapia (Oreochromis niloticus). The design involves treatments with varying concentrations of synthetic waste based on lead nitrate $[Pb(NO_3)]$ on Nile tilapia and subsequent analysis of Pb content in gills and muscle tissue after specific maintenance periods. The resulting data were statistically analyzed to evaluate the relationship between maintenance duration and Pb accumulation levels. Waste sampling was conducted at three discharge points of hospital wastewater flowing downstream in the Watershed (DAS) area of Jember. The analysis of Pb content and treatments on Nile tilapia were performed in a laboratory equipped with facilities for sample preparation, chemical digestion, and Atomic Absorption Spectroscopy (AAS) analysis. The study was carried out over 10 months, encompassing preparation, sampling, treatment, analysis, and data processing stages. The aquatic biota used as bioindicators consisted of Nile tilapia (Oreochromis niloticus), commonly found in freshwater ecosystems, particularly around watersheds exposed to pollutants. The population of wastewater consisted of hospital discharge flowing into the watershed. The fish samples comprised 35 Nile tilapia with an estimated age of ± 1 month, body lengths of 8–12 cm, and weights of 12–15 grams, selected purposively. Hospital wastewater samples were collected from three discharge points (upstream, midstream, downstream) using the grab sampling method with sterile containers to represent variations in location.

The research procedure began with the sample preparation stage, which included the preparation of Nile tilapia specimens and hospital waste. 35 Nile tilapia (Oreochromis niloticus) specimens were selected as bioindicators. The fish was about one month old with a body length of 8-12 cm and a 12-15 grams weight. The organs observed were gills and muscle tissue. Meanwhile, hospital effluent samples were collected from sewers at three stations that flow into the downstream river. These wastes were stored in sealed containers to analyze heavy metal content, particularly lead (Pb). The test used lead nitrate solution $Pb(NO_3)_2$ because nitrate is easily soluble in water. The next stage was sample treatment, where Nile tilapia were exposed to various concentrations of Pb pollutants with different exposure durations. Hospital effluents from the three discharge stations were used directly, with the fish kept in an environment containing these effluents for seven days. The variations of Pb pollutant concentrations and exposure durations are presented in Table 1.

Table 1. Variation of Pb Pollutant Concentrations with Different Exposure Durations

The last stage is sample measurement. The gill organs and meat of tilapia fish were cleaned using deionized water and dried at 105ºC for 24 hours. This procedure yielded the dry weights of the gill organs and meats. Each sample, weighing 0.5 g, was placed in a digestion flask and treated with 10 mL of 65% $HNO₃$ solution in a fume hood overnight. Following this, the resulting organic solution was heated at $100\degree$ C for 15 minutes, and then the temperature was gradually increased to 200 \degree C until the solution became turbid. Subsequently, 1 mL of 70% perchloric acid solution was added to the turbid solution, and it was heated at 100ºC until the solution became clear. In the final step, after achieving a clear solution, 4 mL of deionized water was added, and the mixture was boiled for 5 minutes. For analysis preparation, the solution was diluted with an additional 10 mL of deionized water. Atomic Absorption Spectroscopy (AAS), including Vapor Generation Analysis (VGA), Graphite Furnace Analysis (GTA), and Flame AAS, was employed to determine the heavy metal content using a wavelength of 283.3 nm

The Atomic Absorption Spectroscopy (AAS) instrument can detect the presence of metals in a material according to Beer's Law. This law states that the absorbance of light is directly proportional to the concentration of metal elements, thus providing the concentration of heavy metals. The data obtained from this study are quantitative and are used to identify the presence of heavy metals in the gill organs and meats of tilapia. These data will be plotted on a graph with the duration of maintenance on the x-axis and the concentration of heavy metals in the gill organs and meats on the y-axis. This graph will illustrate the changes in heavy metal content based on variations in concentration and maintenance duration. Additionally, the data will be presented in tabular form and analyzed using statistical tests, beginning with

tests for normality and homogeneity. One-way ANOVA followed by non-parametric Tukey's post-hoc test will be employed for further analysis. The Instrument Validity Framework are presented in Table 2.

Table 2. Instrument Validity Framework

3. RESULT AND DISCUSSION

Result

Heavy Metal Concentration in River Affected by Hospital Waste

The AAS analysis conducted at three stations in the hospital waste discharge area in Jember revealed the highest concentration of lead (Pb) at 0.054 mg/L. This value exceeds the water quality tolerance limit of 0.03 mg/L for Class II, as stipulated in Appendix VI of Government Regulation No. 22 of 2021 concerning Environmental Protection and Management.

Heavy Metal Content in Tilapia Gill Organs

The heavy metal concentration of Pb are presented in Table 2 and the graph of the relationship between pb concentration and maintenance duration in tilapia gill organs are presented in Figure 1.

Table 2. Heavy Metal Concentration of Pb in Tilapia Gill Organs with Varying Waste Concentrations using AAS

Days	Waste Concentration (ppm)							
		0.2	0.4	0.6	0.8	1.0		
	0.04	0.04	0.04	0.04	0.04	0.04		
	0.04	0.82	0.98	1.08	1.19	1.27		
14	0.05	1.03	1.16	1.22	1.32	1.40		
21	0.04	1.12	1.27	1.31	1.38	1.47		
28	0.05	1.23	1.34	1.40	1.42	1.57		

Figure 1. Graph of the Relationship between Pb Concentration and Maintenance Duration in Tilapia Gill Organs

The graph above illustrates the fluctuating concentrations of Pb observed during testing, which vary with different maintenance durations. These fluctuations are attributed to the varying physiological capacities of tilapia to handle pollutants, influencing the levels of heavy metals absorbed by the fish. The heavy metal concentration of Pb are presented in Table 3 and the graph of the relationship between pb concentration and maintenance duration in tilapia meat are presented in Figure 2.

Days	Waste Concentration (ppm)							
		0.2	0.4	0.6	0.8	1.0		
	0.00	0.00	0.00	0.00	0.01	0.00		
	0.00	0.02	0.01	0.08	0.15	0.17		
14	0.00	0.03	0.06	0.12	0.09	0.10		
21	0.01	0.02	0.08	0.09	0.12	0.25		
28	0.00	0.05	0.02	0.09	0.08	0.21		

Table 3. Heavy Metal Concentration of Pb in Tilapia Meats with Varying Waste Concentrations using AAS

Figure 2. Graph of the Relationship between Pb Concentration and Maintenance Duration in Tilapia Meat

The results of the normality, homogeneity and one-way ANOVA analysis of the heavy metal Pb content in gill organs and meat are presented in [Tabel 4.](#page-4-0) The one way ANOVA test showed that there were significant differences in Pb metal content in the gills and flesh of tilapia fish. the analysis of normality, homogeneity, and one way ANOVA are presented in Table 5, the tukey test results on gill organs are presented in Table 6 and The tukey test results on meat organs are presented in Table 7.

Table 4. Content of The Heavy Metal Pb in The Gills and Meat of Tilapia Fish Based on Varying **Concentrations**

**Significant for α=0.05*

Table 6. Tukey Test Results on Gill Organs with Varying Concentrations of the Heavy Metal Pb

**Significant for α=0.05*

Table 7. Tukey Test Results on Meat Organs with Varying Concentrations of the Heavy Metal Pb

**Significant for α=0.05*

Discussion

Heavy Metal Content in Rivers Exposed to Hospital Waste. The results of AAS analysis at three station points in the hospital waste flow area in the Jember area obtained the highest concentration of Pb metal with a value of 0.054 mg/L. This value has exceeded the water tolerance limit, namely 0.03 mg/L for the class II category according to Lamp. VI PP No 22 of 2021 regarding the Implementation of Protection and PLH (Pemerintah Republik Indonesia, 2021). This finding is consistent with previous research indicating that heavy metals, particularly lead, are frequently detected at high concentrations in wastewater from industrial or healthcare facilities due to suboptimal waste treatment processes that fail to filter heavy metals at the micro or nano levels (Lestari & Susanto, 2019; Susilo & Ahmad, 2020). The concentration of contaminants detected in small amounts (ranging from mg/L to ng/L) can bypass wastewater treatment installations, leading to environmental contamination (Garg et al., 2022; Gomes et al., 2018; Khan et al., 2021). Long-term exposure to heavy metals in contaminated water, even at low concentrations, can lead to cumulative harmful effects on human health, such as damage to the nervous system and internal organs ((EPA), 2020; Setiawan et al., 2021)**.**

Heavy Metal Content in Tilapia Fish Gill Organs. According to the distribution of data presented in [Tabel 2,](#page-3-0) it can be seen that on day 0 an increase in concentration or change was not yet visible because the fish were still in the acclimatization process. During acclimatization, monitoring is carried out on the condition of the fish so that they are able to adapt to the new environment. After the acclimatization process, additional waste concentration variations were given to identify the heavy metal Pb content that would be absorbed in the fish organs for 28 days. The use of tilapia as a bioindicator was chosen based on its young age (2 months) because its body resistance is better to environmental changes compared to tilapia aged 4-5 months (adult). Analysis of metal content in fish was carried out in the gills. Because gills are the first route for pollutants to enter the fish's body, they can be used to determine the level of heavy metal contamination. In a study conducted by other researchers, it was stated that, the highest concentration was recorded in the fins, which was seven times higher than the specified limit (Aliyas et al., 2016; Mendoza et al., 2023; Santi & Arsyad, 2021).

The large amount of metals in waters indicates a buildup of pollutants in the organs of aquatic biota (Maddusa et al., 2017). The metal will bind with other compounds, causing the volume and density to become greater, thus potentially speeding up the sedimentation process. In addition, when the metal concentration in the waters is lower than in the sediment, it is thought that a heavy metal accumulation process has occurred in fish. The ability of aquatic biota to acclimatize toxic metals in waters is also influenced by the type and character of the metal (Cahyani et al., 2017; Clara, 2022). Heavy metal data in the watershed is used as the main supporting data to determine variations in Pb concentration that will be used as a living medium for tilapia. [Figure 1](#page-3-1) shows that heavy metals absorbed in the bodies of living creatures will undergo a bioconcentration process, which is the activity of pollutant substances entering the organism through the respiratory system or direct interaction by diffusion through the skin (meat) and bioaccumulation, where the process of pollutant substances entering the system occurs. fish digestion, because the food consumed is heavily infected with heavy metals. This was also confirmed by

other researchers, that the metal absorption process occurs through the interaction between water and fish skin for the respiratory process and metabolic activities through the food consumed by the fish. The level of metal toxicity in aquatic organisms, the concentration will continue to increase if the waters continue to be polluted (Azizah & Maslahat, 2021; Priatna et al., 2016; Purwanto et al., 2020). After eight weeks the amount of heavy metals accumulated was also found to depend on the concentration of the waste and the length of exposure to the fish.

Heavy Metal Content in Tilapia Fish Organs. In [Figure 2,](#page-4-1) there are differences in the levels of accumulation of heavy metals, such as a Pb concentration of 1.0 ppm on days 7 - 28, experiencing ups and downs in the level of absorption in the organs of tilapia fish, due to the influence of temperature which can affect the living activities of the organism, such as fish diet. If there is an increase in temperature, the fish's appetite will increase, and vice versa if the temperature decreases, diet can affect metabolic processes in the body's organs such as health problems, stress and even death (Purwanto et al., 2020; Sukoasih & Widiyanto, 2017). The toxicity process of fish exposed to heavy metals will be distributed through the walls of the digestive tract into the circulatory fluid which is then deposited in fatty tissue. The metal in the fluid will oxidize and accumulate in the liver and not accumulate properly in meat. The Pb content in meat is associated with the physiological process of fish metabolism, in contrast to the liver and kidney organs which are tasked with detoxification and excretion. Apart from that, the low Pb concentration in meat is because the meat (skin) does not act as an active and main tissue in the metal distribution pattern. According by other researchers explain that, the metabolic system in fish less than 2 months old is still not fully formed, resulting in low levels of metal reduction in the fish's body. This is different from adult fish, where their metabolic processes are much more perfect (Busira et al., 2020; Nurrachmi & Amin, 2010).

Based on the results of the analysis and discussion that have been presented, the content of the heavy metal Pb is greatest in the gills with a maintenance period of 28 days. This proves that the gills are the first organ infected by heavy metals in the water after the skin. This statement is supported by AAS analysis data which states that the Pb content with variations in concentration and length of rearing shows that the longer the fish are exposed to hospital waste containing Pb in the water, the concentration of metal content that accumulates in the organs will also be greater and the results Normality analysis in [Table 5](#page-5-0) shows that the Pb content data in gills and meat is normally distributed ($p > 0.05$). Furthermore, homogeneity shows that the concentration variations are not significantly different ($p > 0.05$). The results of one-way ANOVA in [Table 5](#page-5-0) show a significant difference in Pb content between gills and meat (p < 0.05). Post-hoc analysis using the Tukey HSD test in [Table 6-7](#page-5-1) shows that the Pb content in gills is significantly higher than in meat. These findings indicate that gills are more susceptible to Pb accumulation compared with meat, which may be due to the physiological function of gills as respiratory and excretory organs in fish. The results of measurements of the heavy metal Pb, which were tested using AAS, were also compared with good quality standards according to BPOM regulations (food categories for fish and fishery products), namely 0.20 mg/kg as well as with SNI 7387-2009 for food products with a maximum limit of heavy metal Pb contamination of 0.25mg/kg so that it can be ensured that the heavy metal content in the watershed due to the influence of hospital waste is still within the safe threshold value for activities. domestic, agricultural irrigation and suitable for consumption as a food source for the livestock needs of communities around the watershed (BPOM, 2018; BSN, 2009; Pratiwi, 2020).

The findings of this study contribute significantly to the scientific understanding of lead (Pb) contamination levels in river basins (DAS) exposed to hospital waste, revealing that the concentrations remain below the thresholds established by BPOM and SNI standards. This indicates that hospital waste in the studied area has not yet caused significant negative impacts on water quality for domestic activities, irrigation, or fish consumption as a food source. However, the study has certain limitations, including its limited geographic coverage at three sampling points and its focus solely on Pb without considering other potential contaminants such as Hg, Cd, or Cr, which may also pose a threat to the DAS. Furthermore, the research was conducted during a single time period, without accounting for seasonal variations that might influence heavy metal concentrations. Therefore, the implications of this study not only provide assurance to the local community regarding the safety of fishery products and DAS water but also highlight the importance of sustainable hospital waste management. Recommendations for future research include expanding the study area, analyzing a broader range of heavy metals, conducting long-term measurements to account for seasonal variations, and developing more effective waste treatment technologies. This study opens avenues for further research to identify potential long-term health risks and enhance environmental management to safeguard ecosystems and public health in the vicinity of the DAS.

4. CONCLUSION

This study indicates that Nile tilapia (*Oreochromis niloticus*) reared in river environments contaminated by hospital waste exhibit bioaccumulation of lead (Pb) in various body organs. The Pb concentration in tilapia flesh remains within safe consumption limits according to BPOM standards. However, the Pb concentration in gill tissues exceeds the permissible threshold, rendering this organ unsuitable for consumption. These findings highlight potential health risks that warrant attention, particularly in fish organs that fail to meet food safety standards. Therefore, it is crucial to enhance hospital waste management practices to prevent the accumulation of harmful heavy metals, which pose threats to aquatic ecosystems and public health.

5. ACKNOWLEDGE

Acknowledgment is extended to LP2M for supporting this study through the 2024 Internal Grant Program for Beginner Lecturer Research (PDP) under Contract No. 3014/UN25.3.1/LT/2024. The funding provided has facilitated the successful execution of this research in alignment with the targeted outcomes.

6. REFERENCES

- (EPA). (2020). *Human Health and Ecological Risk Assessment*. U.S. EPA. https://www.epa.gov/risk/humanhealth-risk-assessment
- Alam, Z. F., Concepcion, C. K. V., Abdulrahman, J. D., & Sanchez, M. A. S. (2019). Biomonitoring of water bodies in metro Manila, philippines using heavy metal analysis and erythrocyte micronucleus assay in nile tilapia (Oreochromis niloticus). *Nature Environment and Pollution Technology*, *18*(3), 685–696. https://neptjournal.com.
- Aliyas, S. Ndobe, & Ya'la, Z. R. (2016). Pertumbuhan Dan Kelangsungan Hidup Ikan Nila (Oreochromis sp.) Yang Dipelihara Pada Media Bersalinitas. *Jurnal Sains Dan Teknologi Tadulako*, *5*(1), 19–27. https://doi.org/10.30649/fisheries.v4i1.61.
- Azaman, F., Juahir, H., Yunus, K., Azid, A., Kamarudin, M. K. A., Toriman, M. E., Mustafa, A. D., Amran, M. A., Hasnama, C. N. C., & Saudi, A. S. M. (2015). Heavy metal in fish: Analysis and human health-a review. *Jurnal Teknologi*, *77*(1), 61–69. https://doi.org/10.11113/jt.v77.4182.
- Azizah, M., & Maslahat, M. (2021). Kandungan Logam Berat Timbal (Pb), Kadmium (Cd), dan Merkuri (Hg) di dalam Tubuh Ikan Wader (Barbodes binotatus) dan Air Sungai Cikaniki, Kabupaten Bogor. *Limnotek : Perairan Darat Tropis Di Indonesia*, *28*(2), 83–93. https://doi.org/10.14203/limnotek.v28i2.331.
- Berlianti, N. A., Widodo, C. S., & Juswono, U. P. (2014). Studi Tentang Pengaruh Limbah Pencemar Terhadap Kandungan Radikal Bebas pada Organ Insang Ikan Nila (Oreochromis Niloticus). *Natural B*, *2*(4), 355–359. https://www.semanticscholar.org/paper.
- BPOM, B. P. O. dan M. (2018). *Peraturan Badan Pengawas Obat dan Makanan Nomor 7 Tahun 2018 tentang Bahan Baku yang dilarang dalam Pangan Olahan*. https://peraturan.bpk.go.id/Details/183201/peraturan-bpom-no-7-tahun-2018.
- BSN. (2009). SNI 7387:2009 Batas Maksimum Cemaran Logam Berat dalam Pangan. *Batas Maksimum Cemaran Logam Berat Dalam Pangan*, 1–29. https://sertifikasibbia.com/upload/logam_berat.pdf.
- Busira, J. J. Q. D., Prihatmo, G., & Pakpahan, S. (2020). Kadar Logam Berat Timbal (Pb) pada Ikan Nila (Oreochromis niloticus) di Sungai Gajah Wong, Yogyakarta. *Prosiding Seminar Nasional Biologi Di Era Pandemi COVID-19*, *September*, 372–379. https://doi.org/10.24252/psb.v6i1.15896.
- Cahyani, N., Lumban Batu, D. T. F., & Sulistiono, S. (2017). Heavy Metal Contain Pb, Hg, Cd and Cu in Whiting Fish (Sillago sihama) Muscle in Estuary of Donan River, Cilacap, Central Java. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *19*(3), 267. https://doi.org/10.17844/jphpi.v19i3.15090.
- Clara, J. (2022). Analisis Konsentrasi Logam Berat Kadmium (Cd) dan Timbal (Pb) Pada Air, Sedimen, dan Tiram (Crassostrea sp.) Di Sungai Tapak, Kecamatan Tugu, Kota Semarang. *JFMR-Journal of Fisheries and Marine Research*, *6*(1). https://doi.org/10.21776/ub.jfmr.2022.006.01.7.
- Ermawati, R., & Hartanto, L. (2017). Pemetaan Sumber Pencemar Sungai Lamat Kabupaten Magelang. *Jurnal Sains &Teknologi Lingkungan*, *9*(2), 92–104. https://doi.org/10.20885/jstl.vol9.iss2.art3.
- Garg, S., Chowdhury, Z. Z., Faisal, A. N. M., Rumjit, N. P., & Thomas, P. (2022). *Impact of Industrial Wastewater on Environment and Human Health BT - Advanced Industrial Wastewater Treatment and Reclamation of Water: Comparative Study of Water Pollution Index during Pre-industrial, Industrial Period and Prospect of Wastewater Treatment for Water Resource Conservation* (S. Roy,

A. Garg, S. Garg, & T. A. Tran (eds.); pp. 197–209). Springer International Publishing. https://doi.org/10.1007/978-3-030-83811-9_10.

- Gomes, I. B., Simões, L. C., & Simões, M. (2018). The effects of emerging environmental contaminants on Stenotrophomonas maltophilia isolated from drinking water in planktonic and sessile states. *Science of The Total Environment*, *643*, 1348–1356. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.06.263.
- Guevarra, R. D., Paraso, M. G. V., & Lola, M. S. E. G. (2020). Biomarker evaluation in Nile Tilapia (Oreochromis niloticus) to assess the health status of aquaculture areas in the Seven Lakes of San Pablo. *Philippine Journal of Science*, *149*(3), 833–840. https://doi.org/10.56899/149.3a.11.
- Hama Aziz, K. H., Mustafa, F. S., Omer, K. M., Hama, S., Hamarawf, R. F., & Rahman, K. O. (2023). Heavy metal pollution in the aquatic environment: efficient and low-cost removal approaches to eliminate their toxicity: a review. *RSC Advances*, *13*(26), 17595–17610. https://doi.org/10.1039/d3ra00723e.
- Khalil, C., Al Hageh, C., Korfali, S., & Khnayzer, R. S. (2018). Municipal leachates health risks: Chemical and cytotoxicity assessment from regulated and unregulated municipal dumpsites in Lebanon. *Chemosphere*, *208*, 1–13. https://doi.org/https://doi.org/10.1016/j.chemosphere.2018.05.151.
- Khan, M. T., Shah, I. A., Ihsanullah, I., Naushad, M., Ali, S., Shah, S. H. A., & Mohammad, A. W. (2021). Hospital wastewater as a source of environmental contamination: An overview of management practices, environmental risks, and treatment processes. *Journal of Water Process Engineering*, *41*(1), 101990. https://doi.org/https://doi.org/10.1016/j.jwpe.2021.101990.
- Kirana, G. C., Khairuddin, K., & Yamin, M. (2022). Analyss of Heavy Metal Content of Copper (Cu) in Cork Fish From Rawa Taliwang Lake, West Sumbawa Regency 2021. *Jurnal Biologi Tropis*, *22*(3), 1033– 1039. https://doi.org/10.29303/jbt.v22i3.3957.
- Lestari, R., & Susanto, H. (2019). The Effectiveness of Wastewater Treatment Plants in Removing Heavy Metals. *Journal of Environmental Technology*, *17*(1), 45–55.
- Maddusa, S. S., Paputungan, M. G., Syarifuddin, A. R., Maambuat, J., & Alla, G. (2017). Kandungan Logam Berat Timbal (Pb), Merkuri (Hg), Zink (Zn) Dan Arsen (As) Pada Ikan Dan Air Sungai Tondano , Sulawesi Utara. *Al-Sihah: Public Health Science Journal*, *9*(2), 153–159. https://doi.org/10.24252/as.v9i2.3766.
- Mendoza, L. C., Nolos, R. C., Villaflores, O. B., Apostol, E. M. D., & Senoro, D. B. (2023). Detection of Heavy Metals, Their Distribution in Tilapia spp., and Health Risks Assessment. *Toxics*, *11*(3), 1–17. https://doi.org/10.3390/toxics11030286.
- Mester, T., Szabó, G., Sajtos, Z., Baranyai, E., Szabó, G., & Balla, D. (2022). Environmental Hazards of an Unrecultivated Liquid Waste Disposal Site on Soil and Groundwater. *Water (Switzerland)*, *14*(2). https://doi.org/10.3390/w14020226.
- Noor, S. Y. (2020). Konsentrasi logam berat Kadmium (Cd) dan Timbal (Pb) pada air , sedimen dan Ikan Nila (Oreochromis niloticus Linn .) di Danau Limboto. *Prosiding Seminar Nasional Peningkatan Mutu Pendidikan*, *1*(1), 289–293. https://semnasfkipunsam.id/index.php/semnas2019/article/view/113.
- Nurrachmi, I., & Amin, B. (2010). Kandungan Logam Cd, Cu, Pb dan Zn Pada Ikan Gulama (Sciaena Russelli) dari Perairan Dumai Riau: Amankah Untuk Dikonsumsi? *Jurnal Teknobiologi*, *1*(1), 72–84. https://teknobiologi.ejournal.unri.ac.id/index.php/JTB/article/view/1954.
- Pemerintah Republik Indonesia. (2021). Lampiran VI tentang Baku Mutu Air Nasional PP Nomor 22 Tahun 2021 Tentang Penyelenggaraan Perlindungan dan Pengelolaan Lingkungan Hidup. *Sekretariat Negara Republik Indonesia*, *1*(1), 483. https://jdih.setkab.go.id/PUUdoc/176367/PP_Nomor_22_Tahun_2021.pdf.
- Pratiwi, D. Y. (2020). Dampak Pencemaran Logam Berat (Timbal, Tembaga, Merkuri, Kadmium, Krom) Terhadap Organisme Perairan Dan Kesehatan Manusia. *Jurnal Akuatek*, *1*(1), 59–65. https://doi.org/10.24198/akuatek.v1i1.28135.
- Pratiwi, D. Y., Nugroho, A. P., & Yustiati, A. (2019). Bioakumulasi ion tembaga pada ikan nila (Oreochromis niloticus l.) di Instalasi Pengolahan Air Limbah (IPAL), Bantul. *Jurnal Akuatika Indonesia*, *4*(2), 57– 64. https://dx.doi.org/10.24198/jaki.v4i2.25260.
- Priatna, D. E., Purnomo, T., & Kuswanti, N. (2016). Kadar Logam Berat Timbal (Pb) pada Air dan Ikan Bader (Barbonymus gonionotus) di Sungai Brantas Wilayah Mojokerto The Content of Heavy Metal Lead (Pb) in the Water and Bader Fish (Barbonymus gonionotus) of Brantas River , Mojokerto Region. *Lentera Bio*, *5*(1), 48–53. https://www.semanticscholar.org/paper.
- Purwanto, A. I., Prihatmo, G., & Pakpahan, S. (2020). Kandungan Logam Berat Timbal (Pb) pada Ikan Nila (Oreochromis niloticus) dan Ikan Bawal (Colossoma macropomum) di Sungai Winongo, Yogyakarta. *Sciscitatio*, *1*(2), 70–78. https://doi.org/10.21460/sciscitatio.2020.12.31.
- Rahayu, Y., Juwana, I., Marganingrum, D., & Lingkungan, J. T. (2018). Kajian Perhitungan Beban Pencemaran Air Sungai Di Daerah Aliran Sungai (DAS) Cikapundung dari Sektor Domestik. *Jurnal Rekayasa Hijau*, *2*(1), 61–71. https://doi.org/10.26760/jrh.v2i1.2043.
- Santi, A., & Arsyad, M. A. (2021). Kualitas Air dan Cemaran Logam Berat Merkuri (Hg) dan Timbal (Pb) pada Ikan Nila (Oreochromis niloticus) Hasil Tangkapan dari Waduk Tunggu Pampang Kota Makassar Water Quality and Heavy Metal Contamination of Mercury (Hg) and Lead (Pb) in Tilapia. *Galung Tropika*, *10*(3), 292–303. https://doi.org/10.31850/jgt.v10i3.799.
- Setiawan, A., Riyanto, T., & Santoso, P. (2021). The Impact of Heavy Metal Bioaccumulation on Aquatic Ecosystems and Human Health: A Literature Review. *Journal of Environmental Science and Health*, *39*(4), 183–195. https://doi.org/10.31018/jans.v14i4.3900.
- Shah, M. T. (2020). Determination of heavy metals in drinking water and their adverse effects on human health. A review. *Pure and Applied Biology*, *9*(1), 96–104. https://doi.org/10.19045/bspab.2020.90012.
- Sukoasih, A., & Widiyanto, T. (2017). Hubungan Antara Suhu, pH dan Berbagai Variasi Jarak dengan Kadar Timbal (Pb) pada Badan Air Sungai Rompang dan Air Sumur Gali Industri Sokaraja Tengah Tahun 2016. *Buletin Keslingmas*, *36*(4), 360–368. https://doi.org/10.31983/keslingmas.v36i4.3115.
- Susilo, B., & Ahmad, F. (2020). The Role of Bioindicators in Monitoring Heavy Metal Pollution in River Waters. *Environmental Monitoring Journal*, *22*(3), 102–111. https://link.springer.com/article/10.1007/BF02349387.
- Xu, H., Gao, Q., & Yuan, B. (2022). Analysis and identification of pollution sources of comprehensive river water quality: Evidence from two river basins in China. *Ecological Indicators*, *135*, 108561. https://doi.org/https://doi.org/10.1016/j.ecolind.2022.108561.
- Yuniarti, E., Ramadhani, S., Efitra, E., & Gustiani, W. (2023). *Viamin*. PT. Sonpedia Publishing Indonesia. https://books.google.co.id/books?id=PfbDEAAAQBAJ.
- Zuhairah, Sitompul, E., Sitorus, E., & Silalahi, Y. C. E. (2020). Analisa Cemaran Logam Merkuri Pada Ikan Air Laut Dan Udang Secara Spektrofotometri Serapan Atom (Ssa) Analysis of Metal Mercury Pollution in Seawater Fish and Shrimp. *FARMANESIA*, *7*(2), 15–18. https://e-journal.sarimutiara.ac.id/index.php/2/article/view/3413.