Chronic toxicity of palm oil mill effluent on survival growth rate and survival length rate of zebra fish (*Danio rerio*) larvae

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Abstract. Palm Oil Mill Effluent (POME) presents a significant ecological challenge due to its high organic load and potential toxicity to aquatic ecosystems. This study examines the chronic toxicity of POME on zebrafish (Danio rerio), focusing on its impact on Specific Growth Rate (SGR) and Specific Length Rate (SLR). A Completely Randomized Design (CRD) was employed, involving three treatments : 0 mL/L (control), 0.5 mL/L (10% LC50-96 hours, Treatment A), and 1 mL/L (20% LC50-96 hours, Treatment B). The results showed significant decrease in SGR and SLR across treatments. Specifically, the SGR decreased from 4.40%/day in the control group to 3.33%/day in Treatment A and 2.61%/day in Treatment B. Similarly, the SLR decreased from 1.13%/day in the control to 0.32%/day and 0.35%/day in Treatments A and B, respectively. These findings underscore the ecological risks of POME, highlighting its potential to disrupt aquatic ecosystem health by impairing fish growth. Moreover, they emphasize the importance of improving industrial waste management practices to mitigate environmental harm and promote sustainable aquatic resource management.

1 Introduction

Palm Oil Mill Effluent (POME) is a byproduct of palm oil processing, consists of condensate wastewater (8–12%) and processing water (13–23%). It poses a significant threat to aquatic environments due to its high organic content, low pH, and macro-nutrients such as nitrogen (N), phosphorus (P), and potassium (K). Without proper treatment, POME can severely degrade water quality, contributing to oxygen depletion and disrupting aquatic ecosystems [1]. In Indonesia alone, POME generation reaches approximately 28.7 million tons annually, presenting both challenges and opportunities. While proper management of POME could unlock its potential as a resource, poor handling results in toxic impacts on both the environment and human health [2].

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In Indonesia, the management of POME remains a pressing issue. Despite efforts to mitigate its impact, many palm oil mills continue to rely on open pond systems for POME treatment. While cost-effective, these systems often fail to meet environmental standards due to incomplete degradation of organic matter and the release of untreated effluents into water bodies. More advanced technologies, such as anaerobic digestion, biogas recovery, and constructed wetlands, have shown promise but remain underutilized due to high implementation costs and a lack of regulatory enforcement. The study of POME's impact on aquatic organisms provides critical insights into its ecological risks and underscores the need for more stringent and sustainable waste management practices in Indonesia's palm oil industry.

The high concentrations of organic matter, oils, fatty acids, and phenolic compounds in POME make it particularly hazardous to aquatic ecosystems. Polycyclic Aromatic Hydrocarbons (PAHs), including naphthalene, fluorene, phenanthrene, fluoranthene, and pyrene, have been identified in POME [3]. Suspended solids from POME can clog fish gills, impairing growth and survival, while also reducing light penetration in water bodies, disrupting the photosynthesis of algae and plankton critical for aquatic food webs.

Study have shown that POME contamination can significantly increase fish mortality, as demonstrated by [5], where POME concentrations of 125–128 mL caused 93.12% mortality in tilapia (*Oreochromis sp.*). Furthermore, LC50-96 hours tests on tilapia identified 126.06 mL/L as the concentration at which 50% of the fish population died within 96 hours [7]. These findings underscore the importance of understanding POME's sub-lethal effects on aquatic organisms.

Zebrafish (*Danio rerio*) are increasingly used as a model organism for toxicological research due to their genetic similarity to humans, ease of maintenance, and rapid reproduction [11]. Approximately 70% of human protein-coding genes and 84% of genes associated with human diseases have homologs in zebrafish [12]. Their transparent embryos and fast development make them ideal for studying the physiological and developmental impacts of toxicants [13]. This study leverages the advantages of zebrafish to investigate the sub-lethal effects of POME exposure, providing critical insights into its ecological and toxicological risks.

2 Material and methods

2.1 POME and fish collection

Palm Oil Mill Effluent (POME), obtained from plam oill processing factory and was transported to the Ecology and Botany Laboratory of UIN Ar-Raniry using ground transportation. Prior to use, the POME was stored in sealed containers at temperatures below 4°C to prevent biodegradation. The test organisms included 45 male zebrafish and 15 female zebrafish (*Danio rerio*), sourced from local traders in Banda Aceh. The collected zebrafish were approximately 4–5 cm in length and weighed around 0.40–0.50 grams. Male zebrafish were identified by their slender bodies, non-bulging abdomens, brighter coloration, pale urogenital openings, and sperm release upon gentle pressure. Female zebrafish were identified by their bulging, soft abdomens, reddish urogenital openings, and the release of eggs under gentle pressure [16].

2.2 Experimental design

The study employed a quantitative research approach using an experimental method with a Completely Randomized Design (CRD). The design consisted of three treatments, each

replicated five times. The POME concentrations for each treatment were based on the LC50-96 hours value (5.156 mL/L), as reported in a previous study [17]. The treatments included a control group (0 mL/L), Treatment 1 with a concentration of 0.5 mL/L (10% of LC50-96 hour), and Treatment 2 with a concentration of 1 mL/L (20% of LC50-96 hour).

Natural spawning media consisted of 15 aquariums, each measuring 21.5 × 14 × 18 cm, filled with 3 liters of water, and equipped with aeration. Additionally, kakaban (nylon fiber mats) were provided as spawning substrates for zebrafish eggs. Natural spawning was conducted with varying ratios of females to males (1:3), depending on the readiness of the broodstock [16]. In this study, a 1:3 ratio was used, with one female zebrafish and three males per spawning unit. Five larvae from each container were measured daily from the day of hatching until the end of the larval phase (day 7 after hatching) to assess growth performance. The main parameters measured in this study were Specific Growth Rate (SGR) and Specific Length Rate (SLR), which were calculated using the following equations:

$$SGR(\%/day) = (\ln W_t - \ln W_0 \times 100) / t \quad (1)$$

Where:
Wt: Final weight (g)
W_0: Initial weight (g)
t: Duration of the experiment (days)

$$SLR(\%/day) = (\ln L_t - \ln L_0 \times 100) / t \quad (2)$$

Where :
Lt : Final length (cm)
L0 : Initial length (cm)
t : Duration of the experiment (days)

2.3 Data analysis

SGR and SLR data were analyzed using one-way Analysis of Variance (ANOVA) to evaluate the significance of differences among treatments. A 95% confidence level ($p < 0.05$) was used to determine statistical significance. Post-hoc analysis was performed using LSD test to identify specific differences between treatment groups.

3 Result and discussion

The SLR values ranged from 2.36 %/day to 4.70 %/day. The highest value was observed in the control group at 3.33 %/day while the lowest was found in Treatment B at 2.52 %/day (Figure 3A). Statistical analysis showed a significant decrease in SLR values for Treatment A (0.32 %/day) and Treatment B (0.35 %/day) compared to the control group (1.13%). However, no significant differences were observed between Treatment A and Treatment B. The Specific Growth Rate (SGR) values ranged from 1.54 to 5.18 %/day. The highest value was observed in the control group at 4.40 %/day, while the lowest was found in Treatment B at 2.61 %/day (Figure 3B). Statistical analysis showed a significant decrease in SGR values for Treatment A (0.32 %/day) and Treatment B (0.35 %/day) compared to the control group (1.13 %/day).

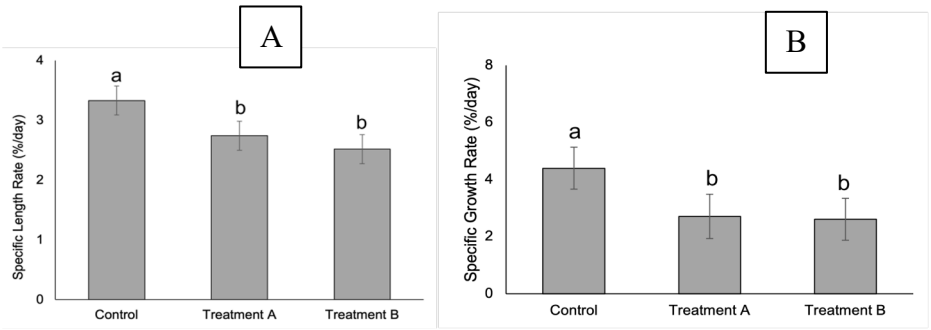

Fig. 1. The values of SLR (A) and SGR (B) of Zebrafish *(Danio rerio)* between treatments are shown, with different letters indicating a significant difference at $P<0.05$.

The observed SLR values in this study align with the standard growth ranges reported by [18] who documented similar growth patterns in laboratory-maintained zebrafish. The control group's higher SLR value [18] falls within the optimal growth range established by [19] for zebrafish under standard husbandry conditions. The significant decreases observed in treatment A (0.32 %/day) and B (0.35 %/day) compared to control (1.13 %/day) mirror the growth inhibition patterns documented by [20] in their comprehensive zebrafish care study.

The reduced SLR values in treatment groups correspond with findings from [21] who reported that alterations in environmental conditions can lead to significant reductions in length growth rates. Our results particularly align with [22], who found that physiological stress can substantially impact growth parameters through epigenetic mechanisms. The similar response magnitude between treatments A and B suggests a common growth inhibition mechanism, a phenomenon also noted by [18] in their feeding frequency studies.

The consistent growth reduction pattern observed in our study reflects the findings of [22] who demonstrated that zebrafish growth responses often show threshold effects rather than linear responses to environmental changes. This is further supported by [19], who established that zebrafish growth parameters typically show clustered responses to different stressors, suggesting common physiological pathways in growth regulation regardless of the specific stress factor.

The SGR values observed in our study fall within the range documented by [23] for laboratory-maintained zebrafish populations. The control group's SGR (4.40 %/day) aligns with optimal growth rates reported by [18], who established baseline SGR values for healthy adult zebrafish under various feeding regimes. The significant reduction in SGR values for treatments A and B corresponds with observations by [20] regarding growth responses to environmental modifications.

The magnitude of SGR reduction in our treatment groups aligns with findings from [22], who documented the physiological mechanisms underlying growth responses in teleost fish. This pattern is consistent with observations by [18], who established that significant deviations from optimal conditions typically result in substantial SGR reductions. The parallel response between treatments, as seen in another study regarding the consistency of growth responses to different types of physiological challenges [21].

The concurrent reduction in both SLR and SGR values suggests a systemic impact on growth, a pattern also documented by [24] in their comprehensive study of zebrafish growth parameters. This integrated response aligns with findings by [18], who demonstrated that environmental factors typically affect multiple growth parameters simultaneously. The magnitude of growth suppression observed in our study falls within the range reported by

[23] for significant physiological stress responses, suggesting that both treatments induced substantial alterations in the fish's growth physiology.

The growth suppression observed in this study has broader implications for fish populations and aquatic ecosystems. Chronic POME contamination can reduce fish biomass and reproductive potential over time, leading to declines in population sizes. Furthermore, the stunting of fish growth can disrupt aquatic food webs by altering predator-prey dynamics. Smaller fish may be less capable of evading predators, while reduced populations of primary consumers, such as zebrafish, may lead to cascading effects on secondary consumers and algae or plankton populations. Over time, this can destabilize the balance of aquatic ecosystems, potentially resulting in biodiversity loss and compromised ecosystem services, such as water purification and nutrient cycling.

4 Conclusion

This study demonstrates that chronic exposure to POME significantly reduces the growth performance of zebrafish (*Danio rerio*), with lower SGR and SLR observed in treatment groups (0.5 mL/L and 1 mL/L) compared to the control. The results indicate that POME exposure induces physiological stress, likely caused by poor water quality and toxic compounds, leading to impaired growth. To mitigate the environmental impact of POME, industry stakeholders should adopt advanced treatment technologies, such as anaerobic digestion systems, biogas recovery units, and constructed wetlands, which have proven effective in reducing the organic load and toxicity of POME. Studies involving multi-species models and natural ecosystems are essential for understanding the broader ecological consequences of POME exposure.

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References

1. A. Azwir, Analisa pencemaran air sungai Tapung Kiri oleh limbah industri kelapa sawit PT. Peputra Masterindo di Kabupaten Kampar. *Progr. Pascasarjana Univ. Diponegoro* (2006)
2. H. Herniwati, Uji kelayakan limbah cair pabrik kelapa sawit PT. Perkebunan Nusantara II Prafi-Manokwari. *Univ. Negeri Papua* (2012)
3. N. F. A. Rasdy, M. M. Sanagi, W. A. W. Ibrahim, A. Abu Naim, Determination of polycyclic aromatic hydrocarbons in palm oil mill effluent by Soxhlet extraction and gas chromatography-flame ionization detection. *Malays. J. Anal. Sci.* **12**, 16–21 (2008)
4. P. Guedenon et al., Acute toxicity of mercury (HgCl2) to African catfish, *Clarias gariepinus* (2012)
5. I. Adatto, C. Lawrence, L. Krug, L. I. Zon, The effects of intensive feeding on reproductive performance in laboratory zebrafish (*Danio rerio*). *PLoS One* **17(11)**, e0278302 (2022)
6. M. S. Fadil, Kajian beberapa aspek parameter fisika kimia air dan aspek fisiologis ikan yang ditemukan pada aliran buangan pabrik karet di sungai Batang Arau. *Artik. Ilmiah. Progr. Pascasarjana Univ. Andalas Padang* (2011)

7. K. Dabrowski, M. Miller, Contested paradigm in raising zebrafish (*Danio rerio*). *Zebrafish* **15(3)**, 295–309 (2018)
8. S. Zahara, U. Umroh, E. V. A. Utami, Effect of palm oil mill liquid waste disposal on Mabat River Bangka River water quality. *Akuatik J. Sumberd. Perair.* **10(1)**, 21–25 (2016)
9. P. P. B. Ismail, P. Pranoto, Kualitas dan beban pencemaran perairan Waduk Gajah Mungkur. *J. Ekosains* **5(1)** (2015)
10. W. A. Wardhana, Dampak pecemaran lingkungan. *Andi Press, Yogyakarta* (2004)
11. H. Nugroho, M. Pasaribu, S. Ismail, *Biota J. Ilm. Ilmu-Ilmu Hayati*, 96–103 (2018)
12. J.-H. He, J.-M. Gao, C.-J. Huang, C.-Q. Li, Zebrafish models for assessing developmental and reproductive toxicity. *Neurotoxicol. Teratol.* **42**, 35–42 (2014)
13. I. Y. Wiendarlina, N. Herlina, E. Mareta, Toksisitas akut sediaan cair berbasis bawang putih dengan metode zebrafish embryo toxicity. *Fitofarmaka J. Ilm. Farm.* **12(1)**, 78–88 (2022)
14. S. Basu, C. Sachidanandan, Zebrafish: A multifaceted tool for chemical biologists. *Chem. Rev.* **113(10)**, 7952–7980 (2013)
15. N. Khosim, H. Latuconsina, R. A. Suhada, Perkembangan embrio dan rasio penetasan telur ikan zebra *Danio rerio* (*Hamilton*, 1822). *Punten Batu Fishing Installations* (2023)
16. J. Karga, S. C. Mandal, Effect of different feeds on the growth, survival, and reproductive performance of zebrafish (*Danio rerio* Hamilton, 1822). *Aquac. Nutr.* **23(2)**, 406–413 (2017)
17. C. Lawrence, J. Best, A. James, K. Maloney, The effects of feeding frequency on growth and reproduction in zebrafish (*Danio rerio*). *Aquaculture* **368**, 103–108 (2012)
18. C. Harper, C. Lawrence, *The Laboratory Zebrafish*. *CRC Press* (2016)
19. A. Avdesh, M. Chen, M. T. Martin-Iverson, A. Mondal, D. Ong, S. Rainey-Smith, R. N. Martins, Regular care and maintenance of a zebrafish (*Danio rerio*) laboratory: An introduction. *J. Vis. Exp.* **69**, e4196 (2012)
20. C. Singleman, N. G. Holtzman, Growth and maturation in the zebrafish, *Danio rerio*: A staging tool for teaching and research. *Zebrafish* **11(4)**, 396–406 (2014)
21. C. Best, H. Ikert, D. J. Kostyniuk, P. M. Craig, L. Navarro-Martin, Epigenetics in teleost fish: From molecular mechanisms to physiological phenotypes. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **224**, 210–244 (2016)
22. J. Nowosad, D. Kucharczyk, K. Targońska, Enrichment of zebrafish *Danio rerio* (*Hamilton*, 1822) diet with polyunsaturated fatty acids improves fecundity and larvae quality. *Zebrafish* **14(4)**, 364–370 (2017)
23. A. P. Dammski, B. R. Müller, C. Gaya, D. Regonato, *Zebrafish: Manual de Criação em Biotério*. *Univ. Fed. Paraná* (2011)