Chronic effects of palm oil mill effluent on the hepatosomatic index of zebrafish (*Danio rerio*)

Ilham Zulfahmi[1*], *Agung* Setia Batubara[2], *Rizwan* Ali[3], *Ayu* Wulandari Nasution[3], *Bela* Puspita[3], and *Badratun* Nafis[4]

[1]Department of Fisheries Resources Utilization, Faculty of Marine and Fisheries, Syiah Kuala University, Banda Aceh, 23111, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan. Kota Medan 20221 Sumatera Utara, Indonesia.
[3]Department of Aquaculture, Faculty of Marine and Fisheries, Syiah Kuala University, 23111, Indonesia
[4]Department of Biology, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Banda Aceh, 23111, Indonesia

Abstract. Palm Oil Mill Effluent (POME) is a significant environmental pollutant that adversely affects fish physiology, particularly liver function, which plays a critical role in detoxification and metabolism. The hepatosomatic index (HSI) is a reliable biomarker for assessing liver health under environmental stress. This study evaluated the effects of chronic POME exposure on the HSI of zebrafish (Danio rerio) to understand their physiological adaptations to POME contamination. A Completely Randomized Design (CRD) was applied, with three treatments (0 mL/L as control, 0.5 mL/L, and 1 mL/L) and four replicates. Statistical analysis using one-way ANOVA revealed no significant differences among treatments ($p > 0.05$), with HSI values ranging at 0.175–0.180 by day 28. Despite fluctuations during the study, such as an initial 20% increase in HSI in treatment A by day 7, the results indicate adaptive hepatic responses without significant toxic effects at the tested concentrations. These findings highlight the resilience of zebrafish to low concentrations of POME, underscoring the importance of HSI as a biomarker for sub-lethal toxicant effects. This study contributes to aquatic pollution management by providing insights into the ecological risks associated with POME and emphasizing the need for stricter wastewater discharge standards and policies to safeguard aquatic ecosystems.

1 Introduction

The palm oil industry is one of Indonesia's largest agro-industrial sectors, contributing significantly to the economy. However, this industry also generates considerable volumes of liquid waste, known as Palm Oil Mill Effluent (POME). POME contains high

* Corresponding author : ilham.zulfahmi@usk.ac.id

concentrations of organic matter, chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS), oils, and fats, all of which can severely pollute aquatic ecosystems if improperly treated [1]. Each ton of fresh fruit bunches (FFB) processed produces approximately 0.7–1 m³ of POME, highlighting the potential environmental risks [2]. When discharged untreated, POME alters water quality and poses significant threats to aquatic organisms, disrupting physiological processes and ecosystem stability. Untreated POME can reduce dissolved oxygen levels in water bodies, leading to hypoxia and impairing the survival of aquatic organisms. Furthermore, its high nutrient content can cause eutrophication, promoting algal blooms that disrupt aquatic food webs.

Zebrafish (*Danio rerio*) have become a widely used model organism in aquatic toxicology due to their genetic similarity to higher vertebrates, including humans, small size, rapid life cycle, and ease of maintenance [3]. The zebrafish liver is a critical organ for xenobiotic metabolism and detoxification, making it a primary focus in toxicological studies. The hepatosomatic index (HSI), defined as the ratio of liver weight to body weight, serves as a reliable biomarker for evaluating the physiological condition of fish and detecting pollutant exposure in aquatic environments [4]. Alterations in HSI can indicate adaptive responses of the liver to environmental stress, such as hypertrophy, hyperplasia, or tissue degradation due to pollutant exposure.

Despite its importance, studies investigating the impact of POME on the HSI of zebrafish under chronic exposure conditions remain limited. Sub-lethal concentrations of POME have been reported to cause histopathological changes in fish livers and disrupt metabolic functions [5]. Moreover, pollutants with high organic content can induce oxidative stress, further influencing liver physiology and HSI values [6]. This study aims to evaluate the effects of chronic POME exposure on the HSI of zebrafish, providing insights into the biological responses to environmental stressors and the potential risks posed by industrial waste pollution.

2 Material and methods

2.1 POME and fish collection

Palm Oil Mill Effluent (POME) was obtained from the primary sedimentation pond of palm mill factory, located in Aceh Province. Before use, the POME was stored at a temperature below 4˚C in a sealed container to prevent biodegradation. A total of 540 zebrafish (*Danio rerio*) with uniform coloration (pink) were acquired from ornamental fish vendors in Banda Aceh. The fish used were adult zebrafish, identified by their larger body and abdomen size. The zebrafish were approximately measuring 3.5–4 cm in length and weighing 0.5–0.7 g. Before the experiment began, the fish were acclimatized in a container box for 7 days to minimize excessive stress during maintenance. During acclimatization, the fish were fed commercial pellets twice daily to satiation.

2.2 Experimental design

This study employed a quantitative approach with an experimental method designed using a Completely Randomized Design (CRD) with three treatment levels and four replicates. The POME concentrations used in the chronic toxicity test were determined based on the LC50-96 hours value. The treatments included a control (0 mL/L), a low concentration (0.5 mL/L or 10% LC50-96 hours), and a high concentration (1 mL/L or 20% LC50-96 hours). The experiment was conducted using 12 aquariums, each containing 45 zebrafish in 5 liters of water, enabling comprehensive biological response observations to POME exposure on a laboratory scale.

The experiments were carried out in aquariums measuring 20 × 20 × 20 cm^3, each filled with 6 liters of water. Observations were conducted on 30 zebrafish per experimental unit, with measurements of liver weight and body weight performed on days 0, 7, 14, and 28. The data collected were analyzed using one-way Analysis of Variance (ANOVA) with a 95% confidence level, utilizing SPSS version 26. The results were presented as means and standard deviations to evaluate the significance of differences among treatments.

3 Result and discussion

Observations of the hepatosomatic index (HSI) of zebrafish over a 28-day exposure period revealed fluctuations across all treatments. At the beginning of the study (day 0), the highest HSI value was recorded in Treatment B (0.205), followed by the control group (0.185) and Treatment A (0.170). An increase in HSI was observed on day 7 for Treatment A, reaching 0.210, while the control group and Treatment B showed minimal changes. The increase in HSI during the first week of toxicant exposure represents an initial adaptive response of the liver [5].

Observations on day 14 revealed a decline in HSI values for both treatments, with the control group showing the highest value (0.190), followed by Treatment A (0.180) and Treatment B (0.170). This decrease in HSI values may indicate the ability of the fish to adapt to POME exposure. The reduction in HSI after the initial adaptation period reflects a homeostatic mechanism to regulate liver metabolic functions under environmental stress [5].

At the end of the study (day 28), HSI values tended to stabilize, showing no significant differences among treatments, with values ranging between 0.175 and 0.180. This stabilization indicates that homeostasis in the zebrafish liver had been achieved. Zebrafish can develop adaptive mechanisms to chronic exposure to organic pollutants through the regulation of detoxification enzymes and antioxidant systems [5].

Comparisons among treatments showed that exposure to the tested POME concentrations did not cause significant changes in HSI. In contrast, other studies have reported significant increases in HSI for fish exposed to industrial waste. This difference could be attributed to the relatively low toxicant concentrations used in this study, which were only 10% and 20% of the LC50-96 hours value.

The lack of significant changes in HSI in this study can be explained by several factors. First, the POME concentrations used (0.5 mL/L and 1 mL/L) may still be within the tolerance range for zebrafish. Significant changes in HSI typically occur at higher toxicant concentrations (>30% LC50). Additionally, POME that has undergone treatment processes has lower toxicity due to the degradation of most organic pollutants.

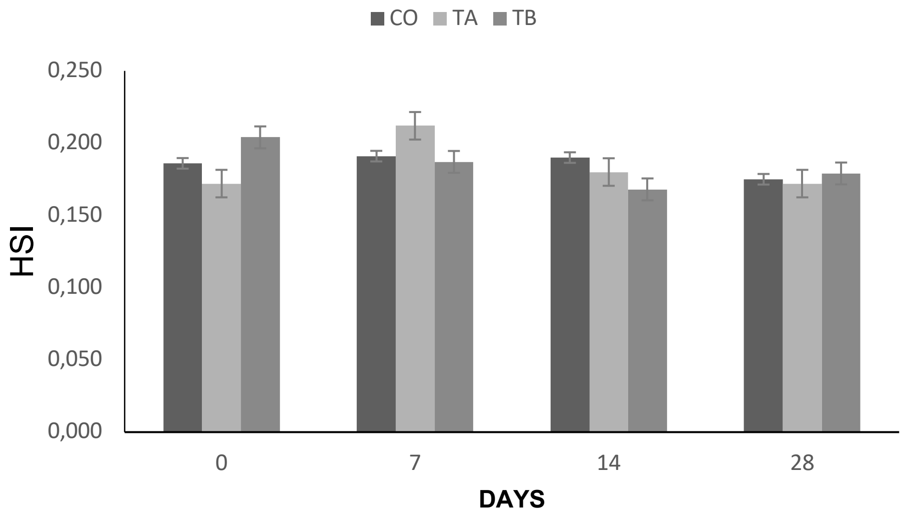

Fig. 1. Hepatosomatic index of Zebrafish in each treatment.

A third factor that may explain the non-significant results is the zebrafish's high adaptive capacity. Zebrafish possess efficient detoxification mechanisms and can adapt well to low concentrations of organic pollutants. Biological responses to toxicant exposure can vary depending on environmental conditions, nutritional status, and other internal factors [7].

Although no significant changes in HSI were observed, the fluctuations in values still indicate a physiological response to POME exposure. Minor changes in HSI can reflect liver metabolism activity and detoxification function, even if they do not reach levels indicative of organ damage. Further studies with longer exposure durations or higher concentrations may be necessary to observe more pronounced effects.

This study provides valuable information on the tolerance of zebrafish to chronic exposure to low concentrations of POME. These findings can serve as a reference for determining wastewater quality standards and evaluating the ecological risks of POME discharge into aquatic environments. However, it should be noted that the absence of significant changes in HSI does not necessarily indicate the absence of toxic effects on other biological parameters.

4 Conclusion

The results of this study demonstrated no significant differences in the Hepatosomatic Index (HSI) values of zebrafish among treatments during the 28-day exposure to Palm Oil Mill Effluent (POME) ($p>0.05$). Despite fluctuations in HSI values, including an initial increase during the first week and a subsequent decrease in the second week, the HSI values stabilized (0.175–0.180) by the end of the study. This stabilization suggests that low concentrations of POME (0.5 mL/L and 1 mL/L) fall within the physiological tolerance range of zebrafish, enabling adaptive responses through liver homeostasis mechanisms without inducing significant organ damage. These findings highlight the resilience of zebrafish to chronic exposure to low concentrations of POME and underscore the importance of using biomarkers like HSI in evaluating sub-lethal toxicant effects. However, further research is needed to assess the impacts of higher POME concentrations, longer exposure durations, and potential effects on other physiological and molecular parameters to provide a comprehensive understanding of the ecological risks associated with POME discharge.

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