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Effects of Microplastics on the Oxygen Consumption and Histological Changes of the Cultured Nile Tilapia *Oreochromis niloticus*

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ABSTRACT

Microplastic pollution has gained global attention due to its toxic impact, particularly on aquatic organisms. As an omnivorous species, tilapia is susceptible to microplastic (MP) exposure in water. This study analyzed the oxygen consumption rate and histology of systemic organs in tilapia exposed to microplastics. An experimental method with a completely randomized design (CRD) was employed in 12 aquariums (40L) equipped with aeration. There were four treatment groups for MP exposure, each with three replicates: concentrations of 1, 10, 100mg/ L, and a control group without MP. The microplastics used were polyethylene particles sourced from soaked plastic bottles collected over 30 days in the Tello River, Makassar. The assessed parameters included oxygen consumption rate and tissue alterations. Data were statistically analyzed using one-way ANOVA for oxygen consumption, while tissue alterations were described qualitatively. The results indicated that the highest oxygen consumption rate occurred at the 10mg/ L treatment (P<0.05), followed by the 1 and 100mg/ L treatments, with the control group showing the lowest rate. Histological tests revealed alterations, including thinning of the interlamella, fractures and swelling of the secondary lamella, and shrinkage of mucus cells in the gills. In the intestines, alterations included thickening of the muscular and serosa layers. The study concluded that MP exposure significantly affects the oxygen consumption rate, particularly at a concentration of 10mg/ L. Additionally, histological alterations were observed in tilapia exposed to microplastics, with the most severe changes occurring in the gills at a concentration of 100mg/ L.

INTRODUCTION

The demand for plastics has surged dramatically over the past half century. As it is well-known, plastic production from 1950 to 2017 reached 9.2 billion tons and it is estimated that the amount will continue to increase until 2050, reaching 34 billion tons **(Geyer, 2020; El-Naggar, 2024)**. This rise in plastic production significantly affects the amount of waste in the environment because of its predominantly single-use nature **(Ghani *et al*., 2024)**. Plastics possess very durable properties, making them difficult to eliminate from the environment and requiring a long time to decompose **(Obebe &**

Adamu, 2020). Plastics only break down into smaller particles, known as microplastics, thereby classifying them as pollutants in the environment **(Ambarsari & Anggiani, 2022)**.

Currently, microplastics have become a prominent environmental contaminant. In recent years, microplastics have garnered global attention, leading to a rapid increase in research on this topic **(Campanale *et al.*, 2020)**. Microplastics are plastic particles that are extremely small, enabling them to be accidentally ingested by aquatic organisms during feeding **(Li *et al.*, 2021)**. Furthermore, microplastics can be ingested because they resemble food types or prey that have been contaminated by microplastics **(Xiong *et al.*, 2019)**. Microplastics pose a significant threat to the survival of aquatic organisms **(Chae & An, 2017)**, particularly because of their accumulation in the digestive organs. Other toxic effects of microplastics include decreased food intake, slowed growth, oxidative damage, and abnormal behavior **(Li *et al.*, 2021)**. One aquatic organism potentially contaminated by microplastics in aquatic environments is the tilapia **(Al-Fatih, 2021)**.

The tilapia species, *Oreochromis niloticus*, can be used as an early sentinel organism of environmental pollutants **(Agustina *et al.*, 2019)**. In addition to its high tolerance to environmental changes, tilapia are easy to breed and exhibit rapid growth **(Ferreira *et al.*, 2015)**. Tilapia are classified as omnivores **(Tesfahun & Temesgen, 2018)** that inhabit the water column, and detritus is one of their food sources **(Pattirane *et al.*, 2022)**. This characteristic makes them susceptible to the accumulation of pollutants including microplastics **(Yana *et al.*, 2021)**. Compared to herbivorous and carnivorous fish, omnivorous fish have been shown to accumulate more microplastics than herbivores or carnivores **(Mizraji *et al.*, 2017)**.

Microplastics that enter the bodies of fish accumulate in digestive organs such as the intestines and stomach **(Yona *et al.*, 2020)**. This accumulation affects food absorption processes **(Li *et al.*, 2021)**, because the presence of microplastics in digestive organs can create a false sense of satiety, leading to reduced appetite in fish **(Jovanovic, 2017)**. In addition to the digestive organs, gills are also potentially contaminated with microplastics, facilitating water entry and exit during the fish respiratory process **(Su *et al.*, 2019)**. **Yona *et al.* (2020)** identified the accumulation of microplastics in fish gills. More specifically, microplastics have been reported to impact mucus and lamellae, disrupting oxygen diffusion in the gills **(Indrayani *et al.*, 2014)**. Disruption of oxygen diffusion can adversely affect various physiological processes in fish including oxygen consumption **(Prakoso & Chang, 2018)**.

Oxygen is a fundamental component of respiratory processes and, is essential for metabolic functions in the body. An insufficient oxygen supply can influence physiological processes and induce stress in fish **(Prakoso & Chang, 2018)**. Stress resulting from disruptions in the digestive and respiratory organs can impact clinical biochemical indicators such as histology **(Hamed *et al.*, 2021)**. Furthermore, it has been explained that the presence of pollutants can affect the metabolic processes of tilapia,

potentially leading to mortality **(Inayah, 2017)**. Research on changes in oxygen consumption in tilapia due to contamination by pollutants, such as metals and detergents, has been conducted **(Martinez *et al.*, 2013; Inayah, 2017)**. Conversely, data related to the impact of microplastic exposure on changes in oxygen consumption in tilapia are still limited. Based on the aforementioned discussion, it was necessary to conduct a study on the effects of microplastic contamination on oxygen consumption and the histology of the systemic organs of tilapia *Oreochromis niloticus*.

MATERIALS AND METHODS

1. Time and location

This study was conducted between January and May 2024. Plastic bottles were submerged in the Tello River, Makassar, for 30 days to simulate exposure to environmental pollutants. Fish maintenance was performed in the Laboratory of Aquaculture Technology, and sample testing was conducted in the Laboratory of Water Productivity and Quality, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar.

2. Experimental fish and design

A total of 200 tilapia specimens (*Oreochromis niloticus*) were transferred from the UPTD Freshwater Fish Seed Center in the Gowa Regency to the Aquaculture Technology Laboratory at the Faculty of Marine Science and Fisheries, Hasanuddin University. The fish were acclimatized for 24 - 48h and were then placed in maintenance containers, each with a volume of 40 liters (Fig. 1). Each maintenance container was stocked with 10 fish, which were fed twice daily at a rate of 6% of their body weight. Microplastic fragments of polyethylene terephthalate (PET) were derived from the plastic bottles. The plastic bottles were cut into small pieces and ground in a blender.

The study was conducted based on the methodology of **Hamed *et al.* (2020)**, utilizing a completely randomized design with three microplastic treatments and one control, each replicated three times. The fish were maintained for seven days following acclimatization.

3. Observation of oxygen consumption

Dissolved oxygen was measured using the iodometric titration method (modified azide method) based on the Indonesian Standardization 2004. Oxygen consumption was measured based on **Djawad *et al.* (1996)** as follows:

$$\text{Dissolved oxygen (mg/L)} \frac{V \times N \times 8000 \times F}{50}$$

$$F = \frac{\text{Bottle volume}}{\text{Bottle volume} - (\text{volume of reagent } MnSO4 - \text{alkali iodide azide})}$$

Where:

V = mL $Na_2S_2O_3$;

N = normalitas $Na_2S_2O_3$;

8000 = Molecular weight of oxygen (O_2);

50 = Volume of Water Sample for Titration.

Then, the amount of oxygen consumed by the fish was calculated using the following formula:

$$OCR = \frac{\text{Initial DO} - \text{Final DO}}{W \times t} \times V$$

Where:

OCR = oxygen consumption rate (mgO_2/body weight/hour) ;
Initial DO = dissolved oxygen at the beginning of the observation (mg/L);
Final DO = dissolved oxygen at the end of the observation (mg/L);
W = weight of fish (gr);
V = water volume (L);
t = observation duration (hours).

4. Histological structure analysis

Histological observations of the gills and intestines were conducted based on the methodology described by **Espinosa *et al.* (2019)**. Gills and intestines were randomly selected from each treatment group, preserved in 10% phosphate-buffered saline (PBS) for 24h, and washed with physiological saline (0.9% NaCl). The samples were then progressively dehydrated using 70, 80, 90 and 100% ethanol, cleared with xylene, and embedded in paraffin. Subsequently, the tissues were sectioned using a microtome to a thickness of 3-5µm, dewaxed, rehydrated with absolute ethanol, and stained with hematoxylin-eosin (HE). After staining, the samples were examined under a microscope.

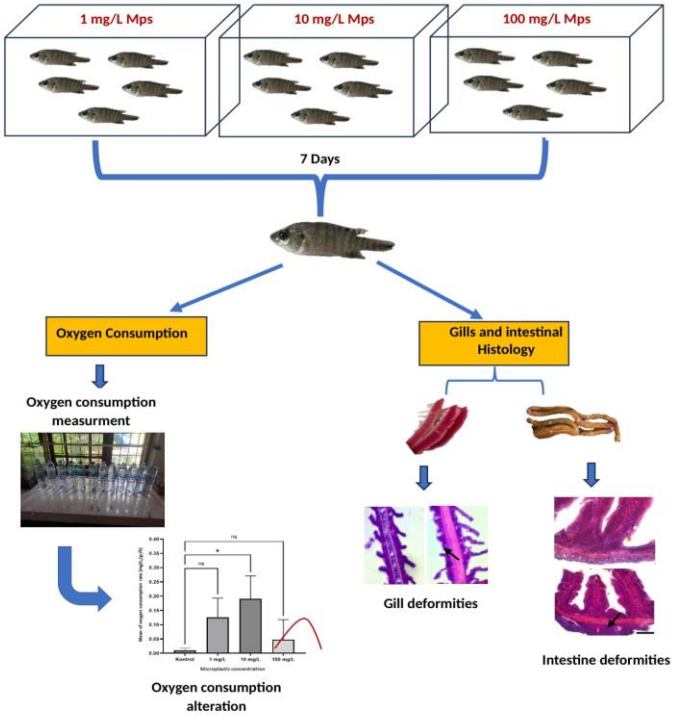

Fig. 1. Experimental design

5. Data analysis

Oxygen consumption data were tested for normality and homogeneity, followed by statistical analysis using one-way analysis of variance (ANOVA) in GraphPad Prism version 8. In contrast, the tissue alterations were analyzed descriptively.

RESULTS AND DISCUSSION

1. Oxygen consumption rate

Oxygen consumption measurements were conducted twice, namely before and after microplastic exposure. The results of the oxygen consumption levels in tilapia can be seen in Fig. (2).

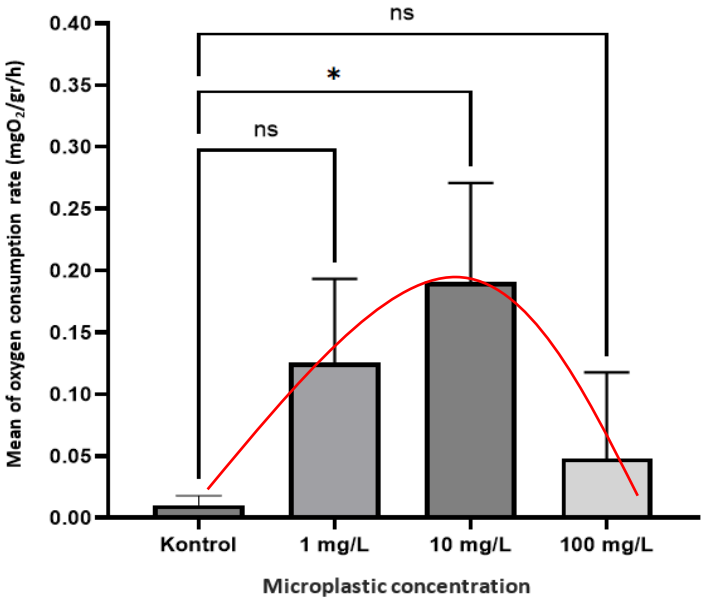

Fig. 2. Oxygen consumption rate of *O. niloticus* exposed to microplastics. Red curve describes the phenomenon of hormetic like-effect

The results indicate that the highest average oxygen consumption level was found in the 10mg/ L treatment, which was 0.190mgO_2/ gr/ h. Apart from the control, the 100mg/ L treatment resulted in the lowest average oxygen consumption level of 0.479mgO_2/ gr/ h, while the 1mg/ L treatment showed an oxygen consumption level was 0.125mgO_2/ gr/ h. In contrast, the average oxygen consumption in the control treatment was 0.010mgO_2/ gr/ h. Statistical testing using one-way ANOVA revealed a significant difference ($P< 0.05$) in the 10mg/ L treatment compared to the control.

2. Histological structure

The results of the histological observations of the gills and intestines of tilapia exposed to different concentrations of microplastics are presented in Figs. (3, 4)

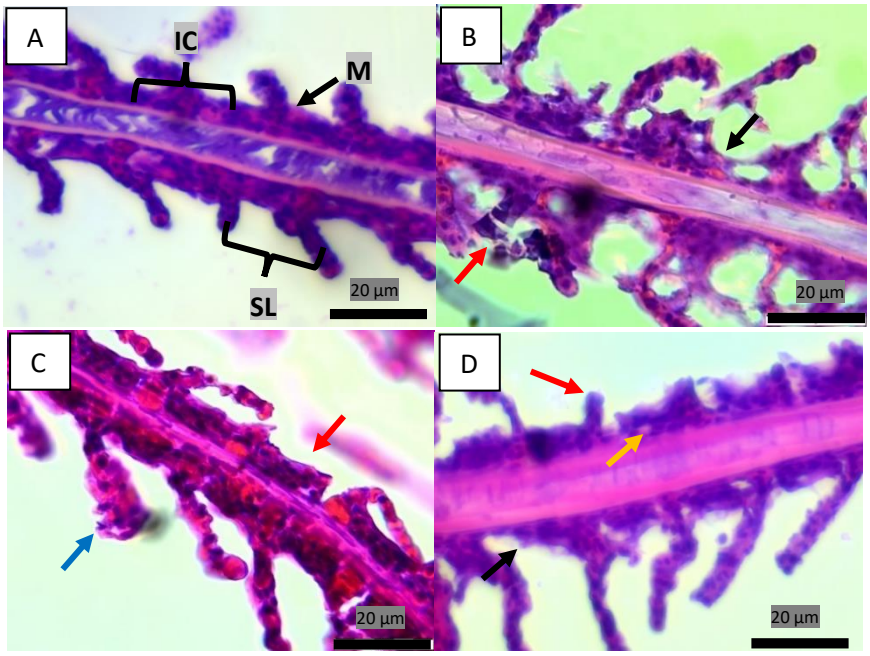

Fig. 3. Histological structure of *O. niloticus' gills* at 100x magnification. The gill tissue consists of interlamellar cells (IS), mucus (M), and secondary lamellae(SL). **(A)** Control; **(B)** 1mg/ L; **(C)** 10mg/ L; **(D)** 100mg/ L. Black arrows indicate interlamellar cell thinning, red arrows indicate secondary lamellar fracture, blue arrows indicate secondary lamellar thickening and yellow arrows indicate mucus shrinkage

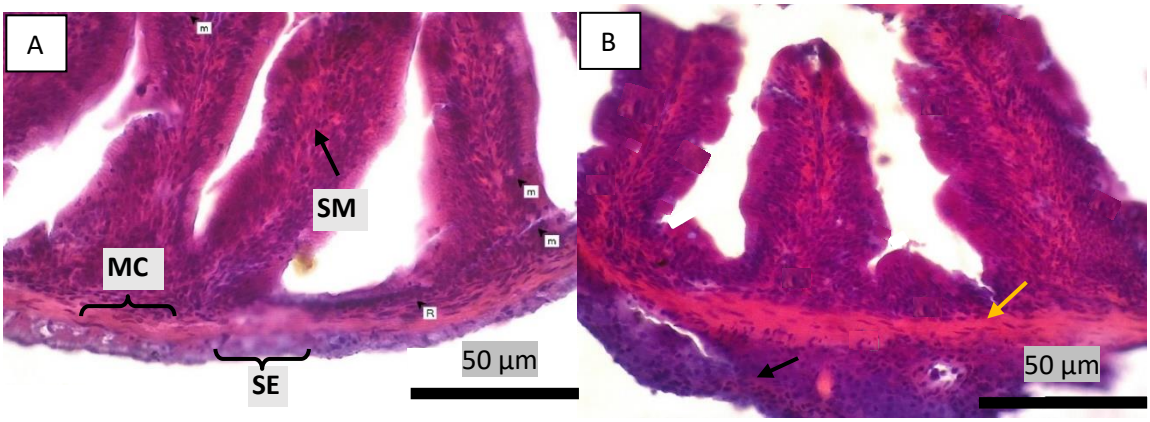

Fig. 4. Histological structure of *O. niloticus*' intestines at 40x magnification. The intestinal tissue consists of serosa (SE), muscularis (MC), and submucosa (SM). **(A)** Intestinal tissue without treatment; **(B)** Intestinal tissue exposed to microplastics. The black arrow indicates thickening of the serosal layer and the yellow arrow indicates thickening of the muscularis layer

The observation results showed that there were differences in the structure of the gill tissue treated with the control. Damage to the histological structure of the gills was assessed based on changes in parts of the gill tissue such as interlamellar cells, secondary lamellae and mucus. The gill tissue of fish treated with 1mg/ L showed thinning of interlamellar cells and breaks in the secondary lamellae (Fig. 3B), while the 10mg/ L treatment showed swelling and breaks in the secondary lamellae (Fig. 3C). In addition, the gill tissues of fish treated at 100mg/ L showed the most significant changes, namely many fractures in the secondary lamellae, thinning of the interlamellar cells and shrinkage of the mucus (Fig. 3D). Damage to the histological structure of the intestine was assessed based on changes in parts of the intestinal tissue such as the serosa, muscularis and submucosa. The observation results showed that there were changes in the intestinal tissue of the control with the intestinal tissue exposed to microplastics. The intestinal tissue of the treated fish showed thickening in the muscularis and serosa layers.

DISCUSSION

1. Oxygen consumption rate

Microplastics are one of the types of contaminants frequently found in aquatic biota and pose serious impacts, particularly on fish **(Subaramaniyam *et al.*, 2023)**. Microplastics that enter the gills and adhere to the skin of fish will affect the rate of oxygen consumption and ion regulation **(Yin *et al.*, 2018)**. The oxygen utilization by fish due to exposure to pollutants such as microplastics is believed to undergo changes during the adaptation, critical, and recovery phases **(Zhang *et al.*, 2017)**. The observations revealed that the highest oxygen consumption level was observed in tilapia treated with 10mg/ L treatment. Subsequently, the pattern of oxygen consumption decreased with an increase in microplastic concentration to 100mg/ L. However, at a concentration of 1mg/ L, a higher oxygen consumption level was observed compared to that at 100mg/ L, but lower than that at 10mg/ L (Fig. 4). In toxicology, this phenomenon is known as hormesis-like effects, a biphasic dose response where the biological effects of a low dose of a stress are opposite to the effects of a high dose of the same stress **(Chapman *et al.*, 2017)**. The type of hormesis observed in fish oxygen consumption is an inverted U-shape, where the effects of microplastic exposure increase with increasing the concentration to a peak, but decrease at higher doses. Fish consuming microplastics at a concentration of 1mg/ L begin to detect the presence of foreign bodies entering their gills, hence their oxygen consumption increases compared to controls; at a concentration of 10mg/ L Mp, the fish have detected the presence of microplastics and are trying to fight the foreign body, therefore metabolism increases, which will affect the increase in oxygen consumption. In contrast to the increased oxygen consumption of fish exposed to 1 and 10mg/ L Mp, the oxygen consumption of fish exposed to 100mg/ L microplastics decreased. This may be because the fish have become highly contaminated that they are

no longer able to combat the contaminants entering through their gills, subsequently the fish's metabolism slows down and oxygen consumption decreases.

The statistical tests indicate that the oxygen consumption level at a concentration of 10 mg/L significantly differed ($P< 0.05$) from the oxygen consumption of fish that were not exposed to microplastics. It is suspected that oxygen conversion in fish contaminated with microplastics drastically increases during critical periods as an oxidative response. Previous research has shown that the rate of oxygen uptake in stressed fish undergoes significant changes owing to microplastic pollution **(Yin *et al.,* 2019)**. Subsequently, fish will become weakened, and oxygen utilization will drastically decrease through the electron transport chain (ETC) after the peak of the stress phase **(Sies, 2015)**. At the cellular level, microplastic contamination causes an increase in lipid peroxidation, thereby increasing oxygen uptake **(Chen *et al.,* 2021)**. **Hasan *et al.* (2024)** further suggested that microplastics act as disrupt mitochondrial function, leading to suboptimal oxygen uptake. Additionally, it is equally important to note that microplastics also serve as carriers of micropollutants such as pathogenic bacteria, which cause oxidative stress in fish. The effects of various pathogenic substances on aquatic organisms can be assessed using indicators of oxygen uptake **(Buwono *et al.,* 2022)**. This aspect is a crucial physiological mechanism in regulating the metabolism of organisms, making the determination of oxygen consumption levels essential **(Rios-Fuster *et al.,* 2021)**. Other reports indicate that exposure to polyethylene MP causes an increase in the rate of oxygen uptake and aerobic metabolic disturbances due to stress that triggers increased metabolic activity **(Oliveira *et al.,* 2013; Yin *et al.,* 2018)**. **Hawke *et al.* (2024)** reported an increase in oxygen consumption in marine fish after exposure to polyethylene MP during a critical period and a slowing of cognitive responses. They go on to explain that there is a delay in response because microplastic ingestion causes chemical and physical stress, thus oxygen is used extensively in the recovery process. Long recovery times require more oxygen, slowing down the fish's ability to respond.

The mechanisms of microplastic (MP) exposure in fish and shellfish are explained by **Oliveira *et al.* (2019)**, who found that MP exposure induces neurotoxicity in the brain, leading to changes in neural and physiological processes that disrupt behavior. Additionally, microplastics have been shown to cause delays in brain activity in teleost fish **(Oliveira *et al.,* 2013)**. These delays may impair the transmission of information through cellular sensors, resulting in slowed locomotor responses and reduced systemic organ function. **Killen *et al.* (2015)** reported that shifts in energy reserves can make fish lethargic during maintenance, a consequence of altered oxygen uptake in the respiratory process. The energy utilized by fish for activity is influenced by the rate of oxygen consumption, which acts as an antioxidant defense and increases during MP exposure **(Campos *et al.,* 2021)**.

Gills are the main respiratory organs, where water enters and exits to take up oxygen and release carbon dioxide. The oxygen consumption of fish is closely related to

the structure of the gill tissue. Damage to gill tissue reduces the efficiency of the gill function in absorbing oxygen. This leads to an increase in oxygen consumption to meet metabolic needs. The greater the damage to the gill tissue, the greater the fish's oxygen consumption, but under certain conditions fish will adapt so that oxygen consumption decreases. This study investigates the oxygen consumption rates of cultured tilapia, which are primarily influenced by water quality, as noted by **El-Hack *et al*. (2022)**. Variations in water quality can impact the mobility of pollutants, subsequently affecting the oxygen consumption of fish **(Lécrivain *et al*., 2021)**. As a limitation of this study, water quality measurements as supporting data are required.

2. **Histological structure**

Histological images of the gill tissue showed thinning of the interlamellar cells, thickening of the secondary lamellae, fractures in the lamellae, and reduction in mucus. This is consistent with the report of **Wang *et al.* (2019)** elucidating that microplastics entering the gills can cause structural damage to the gill filaments, leading to looseness and histological changes such as abscesses or fractures in the secondary lamellae and thickening of the tips of the secondary lamellae. **Guerrera *et al.* (2021)** also noted that microplastics can induce epithelial damage in gill tissues, such as epithelial detachment and swelling in the secondary lamellae. Thinning of interlamellar cells is caused by the damage and shedding of epithelial cells **(Limonta *et al.*, 2019)**. This can lead to a decrease in gas exchange efficiency, resulting in metabolic stress and disruption of gill function **(Yang *et al.*, 2021)**. Fractures in the secondary lamellae may affect the respiratory processes and facilitate the entry of foreign substances into the gills. The results of this study are similar to those of **Karanjkar and Deshpande (2020)**, who reported the presence of fractures in the secondary lamellae of freshwater fish gills. A reduction in mucus weakens the protective system and increases the risk of pathogenic infection. This statement is supported by **Chen *et al.* (2023)**, who emphasized that mucus plays a vital role in the gills as a chemical barrier.

Microplastics trapped in the gills cause fragmentation of the filaments owing to the direct contact between the gills and microplastic particles. The relationship between the penetration of microplastics and the resulting impact is determined by the size and shape of microplastics **(Guerrera *et al.*, 2021)**. Furthermore, fish exposed to fragment-type microplastics exhibit the highest severity of impact compared to other types because the irregular edges of the fragments increase abrasiveness, damaging the gills **(Jabeen *et al.*, 2018)**.

Microplastics are detected everywhere, with various causes ranging from anthropogenic activities to high levels of microplastic pollution, posing a threat to the biota in aquatic environments **(Zhang *et al.*, 2017)**. Microplastics can have both physical and chemical impacts on the organisms that ingest them **(Jeong *et al.*, 2024)**. Histological approaches represent one method that can be used to understand the biological mechanisms underlying microplastic toxicity **(Hamed *et al.*, 2021; Hossain *et al.*, 2022;**

Ali *et al.*, 2023). According to **Muhib and Rahman (2024)**, microplastics accumulate more in the intestines than in the gills; however, the size of microplastics in the gills is larger than that in the intestines. Histologically, fish exposed to microplastics exhibit changes in the gill epithelium, increased neutrophil adhesion, partial fusion of secondary lamellae, and hypersecretion of mucus **(Limonta *et al.*, 2019)**.

These findings revealed changes in the gill and intestinal tissues due to microplastic exposure. Similar findings were reported in the study of **Limonta *et al.* (2019)**, who observed tissue alterations in the gills and intestinal epithelium of the zebrafish exposed to microplastics. **Lei *et al.* (2018)** stated that, overall, histological examination of exposed fish could elicit a strong inflammatory response in the target tissues.

Histological observations of the intestinal tissue revealed thickening of the muscularis and serosa layers. Both layers play crucial roles in the digestion process. The muscularis is involved in peristaltic movement of the intestines, whereas the serosa serves as a protective layer against friction with other organs **(Mcquilken, 2023)**. The thickening of the muscularis and serosa layers is attributed to the increased effort of the fish intestines to digest food. Microplastic particles entering the intestines are difficult to digest, leading to hypertrophy or thickening of the muscle as an adaptive response, as well as infection or inflammation, which results in muscularis thickening as part of the healing process **(Bigliardi *et al.*, 2017)**. Structural damage to the digestive organs and gills was examined by **Karami *et al.* (2016)**, who noted changes in the digestive tract characterized by a reduced number of goblet cells in the epithelial lining. This is suspected to be related to mucus discharge, indicating a relevant first line of defence against pathogen penetration **(Choi *et al.*, 2018)**. Furthermore, the report stated that exposure to microplastics can reduce defensive responses and allocation of energy to combat pathogen disturbances. Microplastics entering fish organs are identified by phagocytes as foreign substances, prompting these cells to secrete mucus as a defense mechanism. Another study reported that the exposure of fish to microplastics at a concentration of 1000µg/ L resulted in increased mucus secretion in the digestive tract, followed by an increase in pathogenic bacteria in the intestines **(Huang *et al.*, 2020)**.

The small size of microplastics makes them easily ingested by aquatic organisms such as shellfish, fish and shrimp **(Guzzetti *et al.*, 2018; Strungaru *et al.*, 2019)**. Once ingested, microplastics accumulate in the gastrointestinal (GI) tract and impede the digestive system of fish, including the stomach and intestines, thereby reducing appetite **(Wright & Kelly, 2017)**. Tissue damage resulting from microplastic exposure leads to changes in behavior, including alterations in feeding patterns, swimming methods, and foraging strategies **(Liang *et al.*, 2023)**. Furthermore, microplastics induce changes in inflammatory responses and apoptosis. Microplastics do not have acute effects, meaning that they do not cause mortality during fish maintenance and treatment **(Choi *et al.*, 2018)**. Although the doses of microplastics used did not result in death, the observed

damage to the gills and intestines provided an evidence that microplastics pose a serious threat to fish.

CONCLUSION

The research concluded that microplastic exposure affects the oxygen consumption rate of the Nile tilapia (*O. niloticus*), particularly at a concentration of 10mg/ L. Histologically, thinning of the interlamellar cells, thickening, and fracture of the secondary lamellae, as well as reduction in mucus in the gills were all recorded. Conversely, thickening of the muscularis and serosa layers of the intestine was observed. A concentration of 100mg/ L caused the most severe changes, especially in the gills.

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