Penetration of Microplastics (Polyethylene) to Several Organs of Nile Tilapia (*Oreochromis niloticus*)

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Abstract. Microplastics are a severe problem in several countries. Microplastics spread throughout the ecosystem both on aquatic and terrestrial. Those are very difficult to overcome as well as have an impact on the environment and affect food safety. This study aimed to know the penetration of microplastics into several organs in Nile tilapia. The experiment was conducted by given 30 grams of microplastic (a type of polyethylene scrub) into an aquarium (15 L) containing five fish (consumption size) for seven days. The results showed that microplastic penetration occurred in the blood, gills, gonads, intestines, liver, muscles, and stomach. These results explain that during a week of rearing, the fish have been exposed to microplastics. It was very dangerous for human health issues if consumed.

Keywords. Microplastics, Nile Tilapia, Polyethylene

1. Introduction

Since 1970 studies on small plastic fragments (microplastics) have done, but only to the discovery. However, in the 2000s, research on microplastics (MPs) was overgrowing because it significantly impacted the environment [1]. This situation can saw from the many recent discoveries about exposure to MPs in fauna throughout the ecosystem at different trophic levels [2][3]. Initially, many microplastics research from marine biota [4], and now it turns out that many are also reporting in freshwater biota [5], terrestrial animals [6], even to humans [7]. In humans, MPs can interfere with human tissue, which has implications for human health [7][8].

The worst impact that occurs by exposure to MPs is aquatic pollution. It happens because MPs are scattering in the surface, column, and sediment in the marine ecosystems [9]. Indonesia was the second-largest contributor to plastic waste in the world, and the first is China [10]. In Indonesia, research on exposure to microplastics in the coastal and marine has carried out [11][12][13][10], while in freshwater, there is not too much [14]. Research models of exposure to MPs in controlled containers are still rare, only findings from a few aquatic systems. Indonesia is one of the countries that frequently consume freshwater fish. One of the freshwater fish that was often consumed in Indonesia, namely Nile tilapia [15]. It was introduced from Africa [9].

MPs-Cadmium was neurotoxic in fish, causing mass mortality in fish, especially in the juvenile phase [16]. It was very dangerous because the MPs in fish enter the human body directly through the...
bioaccumulation process after the human consumes [7]. The consumption of fish by humans is usually only on meat, but it would not rule out the possibility that other organs are eaten as well [15]. Therefore this study aims to know the penetration of MPs into fish organs, relation to food safety.

2. Materials and Methods

2.1 Experimental designs

Based on the modified previous research, this experiment exposed MPs to consumption-sized Nile tilapia [16]. First, five fish (F1, F2, F3, F4, and F5) was acclimatized for 24 hours. Furthermore, the fish are placing in an aquarium container measuring 30 x 30 cm with a volume of 15 litres. The thirty grams of MPs (a type of polyethylene) are then added in an experimental container containing 5 fish, to see the penetration of microplastics against the tilapia organ. Fish rearing carried for seven days by giving feed twice a day (ad libitum). After seven days, the fish was surgically, and some of its organs (bloods, gills, gonads, intestines, liver, muscles, and stomach) were taken for observation.

2.2 Materials

Nile tilapia obtained from fish farmers in Pandeglang, Banten, Indonesia. The size of the fish used was a measure of consumption by length about 12.5 – 14.0 cm and weight about 35.1 – 47.0 gr. The chemical used in this study were KOH (Merck), H\textsubscript{2}O\textsubscript{2} (Merck), and aquadest.

2.3 Extraction of MPs

After seven days of rearing, the blood, gills, gonads, intestines, stomach, liver, and muscles were taken for extraction. Then, the organs were added 10% KOH solution until submerged (approximately 3 times from the tissue volume) to destruct the fish organs (organic matter). Incubation is carried for 24 hours at 60°C. During this process, if the organs are not destroyed, so 5 ml of 30% H2O2 solution is added, then incubated for 24 hours at room temperature. Subsequently, the extraction results were filtered using filter paper (Whatman 42, pore size 2.5 μm) [17]. Lastly, the filtered sample was dried in an oven at 70°C for 24 hours [18].

2.4 Observation of MPs

The identification of MPs used an ocular microscope (Olympus CX-21) magnifies 40 x 10 [18]. The sample on the dried Whatman paper was transferred to a petri dish to facilitate the identification process, then the presence of microplastics observed [19].

3. Results and Discussion

Based on the results of the study, it was recognised that MPs expose all fish organs (F1 –F5) for seven days of rearing (Table 1). The organs observed were blood, gills, gonads, intestines, liver, muscles, and stomach (Figure 1). MPs are found in several organs because it can be seen from the presence of MPs in these organs (Figure 1). In this case, the presence of MPs is not calculated in detail, only if it is observed to be present or not (qualitative data). This indicates that each organ observed can be exposed to MPs for seven days of rearing, according to a previous study [21] which stated that juvenile tilapia can be exposed to MPs throughout the body because MPs enter the blood and are delivered throughout the body through the blood.

Observations were conducted on five tilapia fish with a length of 13.36 ± 0.61 cm and a weight of 42.54 ± 4.95 gr. This situation is hazardous for the survival of the fish itself because exposure to MPs can damage fish tissue and can even enter cells with a size of up to 70 μm [20]. Blood is the primary indicator in knowing exposed to MPs in the fish's body. The blood has shown exposed to MPs, due to pumped by the heart and spread throughout the body, so that it was suspected present in several organs [21]. It can be seen from the results of this research that described muscles as exposing to MPs, one of which is due to the blood spread into the body.
On the gills, MPs can attach to mucous membranes. MPs enter the gills through a cavity which is then filtered and settles in the layer due to the presence of mucus [22]. The presence of this mucus was due to stimulation of the fish’s body as a means of self-defence [23]. Besides gills, it turns out that MPs also enter through the gastrointestinal (GI) tract in fish [5]. In this study, MPs were detected in the intestine and stomach. After entering the GI tracts, it will be absorbed into the blood in a small size so that it will be transported, then enter all other organs such as the liver and gonads [24]. In juvenile tilapia, microplastic exposure to accumulate throughout the body, as seen from the MPs markers found in the blood (in the whole body) [21]. Apart from that, this exposure also accumulates in the gut, gills, liver, brain, gonads, and stomach [25][23][26][24].

### Table 1. Penetration of MPs in Nile tilapia

| Material | Length (cm) | Weight (gr) | Penetration MPs in organs |
|----------|-------------|-------------|--------------------------|
|          |             |             | Bloods | Gills | Gonads | Intestines | Liver | Muscles | Stomach |
| F1       | 12.5        | 35.1        | +      | +     | +      | +          | +     | +       | +       |
| F2       | 14.0        | 45.7        | +      | +     | +      | +          | +     | +       | +       |
| F3       | 13.0        | 39.9        | +      | +     | +      | +          | +     | +       | +       |
| F4       | 13.5        | 45.0        | +      | +     | +      | +          | +     | +       | +       |
| F5       | 13.8        | 47.0        | +      | +     | +      | +          | +     | +       | +       |

Note: present (+), no present (-)

Figure 1. MPs in several organ, (a) blood, (b) gills, (c) gonads, (d) intestines, (e) liver, (f) muscles, and (g) stomach

The effect of MPs for fish health was following the concentration and accumulation time [21]. This condition causes anemia, biochemical perturbations, inhibited AChE activity in the brain, disturbed metabolism in the liver, and MPs-associated HOCs desorb to tissue organisms [8][21][25]. This result is hazardous, considering that people in Indonesia often consumed Nile tilapia [15]. If all parts of the fish are exposed to MPs with a concentration of only 30 grams for seven days of rearing, the fish raised with high MPs waste conditions may be exposed more quickly [27]. Therefore, it will have an impact on health and food safety for humans. Exposure of MPs to humans can occur through tissues and cells [8]. It was evident by the many types of plastics exposed to the human body. One of the plastics that found contaminated is the type of PE (polyethylene). This type of plastic spread throughout the body if released, it was known from the immunological response [6][8].
4. Conclusion
Microplastic exposure occurred in all Nile tilapia organs observed. This exposure is hazardous for food safety and human health indirectly due to accumulation.

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