Microplastics in Tilapia Fish (*Oreochromis niloticus*) Cultured at Magat Dam Reservoir

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Abstract: ***Microplastics (MPs) have become a global concern due to their pervasive presence in the environment, originating largely from discarded plastic fragments. These particles, smaller than 5 mm, may come from various sources including cosmetics, clothing fibers, and tire particles, posing a significant threat to aquatic life and human health. This study focused on the potential presence and ingestion of MPs by Nile tilapia (Oreochromis niloticus) cultured in the Magat Dam Reservoir in Ramon, Isabela, Philippines. The research aimed to determine the presence, type, shape, and color of MPs in Nile tilapia, considering the ecological and health implications of microplastic pollution in aquatic ecosystems and food sources. Sampling was conducted in three distinct zones of the reservoir—upper, middle, and lower. MPs were extracted from their gastrointestinal tracts of the sample fishes using the Wet Peroxide Oxidation technique. The MPs were characterized in terms of type, shape, and color using established criteria. Statistical analysis, employing One - Way Analysis of Variance (ANOVA), was conducted to discern differences in MP distribution across sampling areas. Results indicate a significant prevalence of fiber in the guts of Nile tilapia, suggesting its potential as a significant pollutant in the reservoir. MPs exhibited various shapes including elongated, angular, cylindrical, and degraded, with elongated fibers dominating the gastrointestinal tracts. The color spectrum of MPs ranged from white to black, with transparent MPs being the most abundant. This study underscores the urgent need for mitigation strategies to address microplastic pollution in aquatic environments, emphasizing the importance of sustainable practices to preserve both marine ecosystems and human health. Additionally, it contributes to the fulfillment of Sustainable Development Goals 6 and 14, advocating for clean water, sanitation, and the protection of life below water.***

Keywords: Magat Dam, Microplastics, Nile *Tilapia*, *Oreochromis niloticus*, Reservoir

1. Introduction

Microplastics (MPs) have emerged as a global concern due to their widespread release into the environment, originating mainly from discarded plastic fragments (Yu et al., 2018; Alimba & Faggio, 2019). These tiny plastic grains, classified as particles smaller than 5 mm, can be found in various sources, including cosmetic and personal care products, as well as unintentional sources like clothing fibers and tire particles (Verschoor, 2015).

The pervasiveness of MPs in aquatic environments poses a significant threat to marine life at all levels (Ma et al., 2020; Aragaw, 2021). The ever - increasing use of plastics in daily life has led to millions of tons of plastic waste entering our oceans, soil, freshwater bodies, and sediments, causing severe ecological challenges for different ecosystems and their inhabitants (Ghaffar et al., 2022). Plastics are extensively utilized in diverse industries such as food packaging, construction, automobiles, electronics, sports, healthcare, and household items (Plastics Europe, 2019).

One major consequence of microplastic pollution is its contamination of fish worldwide, subsequently entering the human body through seafood consumption (Sequeira et al., 2020). MPs ingested by fish can accumulate in their gastrointestinal systems, leading to digestive tract obstructions and reduced feeding due to appetite suppression (Lusher et al., 2013; Wright et al., 2013). The presence of MPs in edible fish has been confirmed, and their biomagnification in the food chain allows them to reach human systems (Alfaro - Núñez et al., 2021; Goswami et al., 2020; James et al., 2020), thereby highlighting the potential risks posed by microplastic pollution on marine ecosystems and human health.

On a different note, the production of fishery and aquaculture has undergone an astonishing eightfold increase since the FAO started compiling data in 1950, outpacing human population growth over the past five decades. In 2015, a record - breaking 170 million tons of animal products were produced, not including approximately 29 million tons from farmed aquatic plants. Although fish and fisheries products offer well established health benefits due to their unique nutritional composition, some fish may accumulate harmful contaminants from the environment, depending on the amount consumed (Lusher et al., 2017).

In the Philippines, studies have reported the presence of microplastics in surface water, particularly in locations like the Magat Dam Reservoir, where water quality has been deteriorating due to the intensified disposal and mismanagement of solid and human waste (Chang et al., 2009; Sia et al., 2009). The breakdown of solid wastes, especially plastic, through biological, chemical, and mechanical processes contributes to the potential presence of microplastics in the reservoir.

The Magat Dam Reservoir, covering a vast watershed of 4, 143 square kilometers and fed by eight upstream rivers, plays a vital role in the Municipality of Ramon. This region has capitalized on the rich environment to cultivate *Tilapia*, a fast - growing species known for its adaptability to various environmental conditions, efficient utilization of natural food organisms, and low protein requirement (Wolhfarth et al., 1993). Local fishers construct fish cages in designated zones to facilitate *Tilapia* farming.

*Tilapia*s feed on a variety of natural food organisms, including plankton, aquatic macrophytes, aquatic invertebrates, larval fish, detritus, and decomposing organic matter. With supplemental feeding, these natural food organisms significantly contribute to *tilapia* growth. The species' efficiency in harvesting plankton makes it a filter feeder (Towers, 2005), and it's easy breeding and farming characteristics, as well as its ability to assimilate plant protein, have made it an attractive choice, especially in resource - poor rural areas.

Tilapia holds significant cultural and culinary importance in many Asian countries, including the Philippines and Indonesia, where it has become an integral part of the national cuisine. Its value was recognized by international development agencies, earning it the moniker "Aquatic Chicken" in the 1970s and later becoming known as the "fish of the 1990s. " Presently, *Tilapia* is hailed as the "Food fish of the 21st Century" (Costa - Pierce et al., 2000; Ramnarine et al., 2005). Asian countries, led by China, dominate *Tilapia* production and consumption, with Egypt, Indonesia, Thailand, Brazil, Bangladesh, Vietnam, Colombia, and Malaysia being other major producers.

Furthermore, this study is crucial because it focuses on the Sustainable Development Goal 14: Life Below Water, with a commitment to preserving its marine treasures and combating pollution that threatens their vitality. It encompasses not only preserving marine life but also fresh waters as well. Also, with Sustainable Development Goal 6: Clean Water and Sanitation specifically on the Protection and restoration of water related ecosystems, this entails preserving and restoring wetlands, rivers, lakes, and other water - related ecosystems to enhance their resilience, biodiversity, and ecosystem services. Moreover, ensuring integrated water resources management, that involves adopting integrated approaches to water resources management that consider the interconnectedness 4 of water systems, ecosystems, and human activities, while also promoting transboundary cooperation and sustainable water governance.

Considering the environmental concerns surrounding microplastics in the Magat Dam Reservoir and the significance of *Tilapia* in the aquaculture industry in the area, this study aims to identify the potential presence and ingestion of microplastics by *Tilapias* (*Oreochromis niloticus*) within the reservoir in Ramon, Isabela. This research holds implications for both ecological conservation and human health, given the interconnectedness of microplastic pollution in aquatic ecosystems and food sources.

2. Methodology

2.1 Sampling Area and Sampling Method

There are three sampling sites within the reservoir: one located in the upper portion, one in the midpart, and another in the lower part. At each of these sampling sites, quadrats measuring 1m x 1m was established. From these quadrats, three was randomly selected as sampling plots. In each selected sampling plot, three *Tilapia* fish samples were randomly collected, resulting in a total of 27 *Tilapia* samples taken from the three sampling sites. To ensure the integrity and prevent contamination during transportation, the fish samples were carefully wrapped in aluminum foil and placed in a secure container. The samples were then transported in a cooler with ice, maintaining a temperature range of 0 - 4°C.

2.2 Preparation of the Fish Samples for Analysis

In the laboratory, the fish samples had undergone dissection and comprehensive analysis. Prior to dissection, the weight and length of each fish were measured to obtain data for subsequent examination and evaluation.

2.3 Analysis of MP Accumulation for Fish Samples

In the laboratory, each fish was carefully dissected to extract the entire gastrointestinal tract. The extracted gastrointestinal tract was then placed in a 600 - ml glass 29 beaker and heated on a hot plate at a temperature of 80°C for a duration of 15 minutes. After the heating process, the sample had undergone centrifugation for 20 minutes at a speed of 1000 rpm and at an approximate temperature of 25°C. Next, the dried samples were subjected to Wet Peroxide Oxidation by adding 30% H_2O_2 in 30 ml, and the mixture will be soaked for 24 hours. The soaked samples were filtered using steel sieves with mesh sizes of 1mm and 300μm (mesh size used: 0.80 μm) and washed with distilled water to separate the MP particles from the sample.

The collected residue was then combined with 20 ml of aqueous ferrous (II) sulfate and subjected to heating and stirring at 60°C for 15 minutes (300 rpm). The samples had undergone another filtration step using Whatman Grade No.125mm filter paper and were washed with distilled water. Subsequently, each filter paper with the sample was carefully placed on a petri dish and oven - dried for 4 hours at a temperature of 40°C to remove any remaining moisture. The dried samples were examined under a dissecting microscope at magnifications of 10 - 40x. Any identifiable microplastic particles was photographed and characterized using the modified criteria from Hidalgo - Ruz et al. (2012).

2.4 Data Gathered

The data gathered were the following:
1) Characterization of MPs in the different samples as to type, shape, and color following the criteria used by Hidalgo - Ruz et al. (2012):

Table 1: Criteria for the characterization of MPs in the different samples as to type, shape, and color

Characteristics	Description
Type	plastic fragments, pellets, filaments, plastic films, foamed plastic, granules, and Styrofoam
Shape	*for pellets*: cylindrical, disks, flat, ovoid, spherulites *for fragments:* rounded, subrounded, subangular, angular *general:* irregular, elongated, degraded, rough, and broken edges
Color	transparent, crystalline, white, clear - white - cream, red, orange, blue, opaque, black, gray, brown, green, pink, tan, yellow, and pigmentation

2) The number of MPs in the different samples in terms of type, shape, and color
3) The total amount of microplastic accumulation in fishes

2.5 Statistical Treatment of Data

The One - Way Analysis of Variance (ANOVA) was utilized to determine the differences of MPs in the different sampling areas.

3. Discussion of Results

Type and amount of MPs Detected in the Fish Samples
There are four (4) types of MPs detected from the Nile *tilapia* grown in Magat Dam Reservoir as shown in Table 2. These MPs are fibers, plastic fragments, plastic films and pellets.

Table 2: Types and amount of MPs detected in the Nile *tilapia* fish sample

Type of MP	Mean
Fiber	189.00^a (81.46%)
Plastic Fragments	15.67^b (6.75%)
Pellets	12.67^b (5.46%)
Plastic Films	14.67^b (6.32%)
ANOVA	**

*** - significant at 1% level of significance*
Means having the same letters are not significantly different.

Result of the study suggests that the amount of fiber found in the guts of Nile *tilapia* is significantly higher compared to other types of microplastics (MPs) like plastic fragments, pellets, and plastic films. If fiber is prevalent in the guts of Nile *tilapia* in the Magat Dam reservoir, where they are being reared, it suggests that fiber might be a significant pollutant in the reservoir.

Consistent with previous emerging environmental studies, detected MPs consisted mainly of fibers (Lusher et al.2013; Rochman 2015; Pazos et al.2018). Lim et al. (2022) also claimed that plastic fibers (70.6%) were the most prevalent plastic components ingested by fish. Several studies have documented fiber plastics to be the most prevalent type of plastic in seawater, freshwater, and aquaculture environments (Gago et al., 2018; Wang et al., 2021; Wang et al., 2017; Burns & Boxall, 2018). Current studies have revealed the ubiquity of textile microfibers in the environment, including the atmosphere, different water sources, sludge, sediments of rivers, oceans, and topsoil (Yu et al., 2018; Campanale, et al., 2020; Welsh et al., 2022; Santini et al., 2022). As reported by Liu et al., microfibers are defined as "particles with a diameter less than 50 µm, and length ranging from 1 µm to 5 mm".

However, in this study, the fibers measures more or less 200 µm as shown in Figure 1a. The presence of fiber in the reservoir could stem from various sources. According to Pinlova and Nowack (2023), synthetic textiles are considered a prime source of MP fibers which are a prevalent in MP pollution. Fibers are secondary MP that may be released from synthetic garments during laundering (De Falcoet al., 2019). While the release mechanisms and formation of such MP fibers have been so far mainly studied in connection with laundry washing, there are some studies emerging that describe also other release pathways for MP fibers such as abrasion during wearing (Pinlova &Nowack, 2023).

An experiment illustrated that a single garment could produce >1900 fibers per wash and all garments can release >100 fibers per liter of effluent (Browne, 2011). Similarly, it was estimated that over 700, 000 fibers could be discharged from an average wash load of 6 kg fabrics (Napper & Thompson, 2016). Another source of fiber plastic in the environment could be from the fishery activities. The abrasion of abandoned, lost, or discarded fishing gears has contributed about 18% of the marine plastic debris in the marine environment (Andrady, 2011)

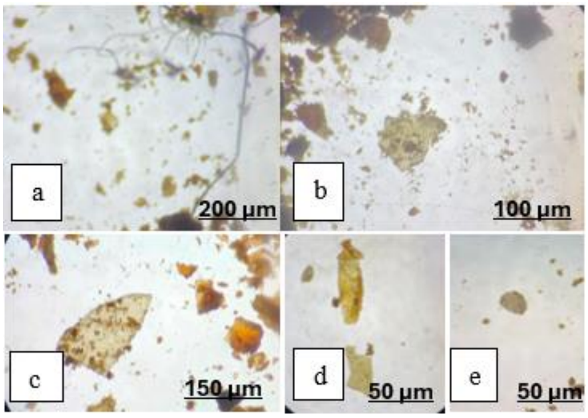

Figure 1: Sample of MPs obtained in the gastro - intestinal tract of *Oreochromis niloticus* under FT - IR Microscope. (a) Fibers, (b and d) Plastic Fragments, (c) Plastic Films, (e) pellet

Shapes of MPs in the Nile *Tilapia*
The MPs in the fish samples obtained from the Magat Dam reservoir contain microplastics (MPs) of various shapes, including elongated, angular, cylindrical, and degraded (Table 3).

Table 3: Shapes and amount of MPs in the fish samples

Shape of MPs	Mean
Elongated	196.00^a (84.48%)
Angular	8.67^b (3.85%)
Cylindrical	13.33^b (5.92%)
Degraded	14.00^b (6.25%)
ANOVA	**

*** - significant at 1% level of significance*
Means having the same letters are not significantly different.

Result of the study shows that significant amount of elongated MPs in Figure 2a are detected in the gastro - intestinal guts (GIT) of Nile *tilapia* fishes. Few of these microplastics are degraded (Figure 2d), cylindrical (Figure 2c), and the least is angular (Figure 2b) (Table 2).

The prevalence of elongated - shaped MPs can be associated with the dominance of fibers in the GIT of Nile *tilapia*. It can be noted that fibers are usually thin and elongated MPs possibly originating from fishing paraphernalia such as nylon nets and lines. Plastic fragments were found to be angular, subangular, with jagged edges or sharp corners, indicating that they are likely to be secondary MPs that had broken off from larger products. Plastic films were observed to be degraded and crumpled or irregular in shape.

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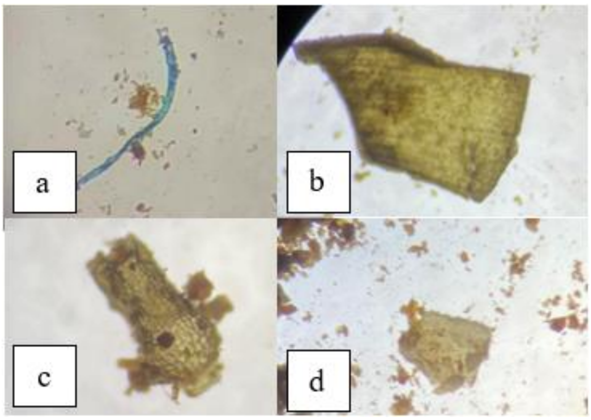

Figure 2: Shapes of the MPs obtained from the GIT of Nile *tilapia* fishes obtained in Magat Dam reservoir (a) elongated, (b) angular, (c) cylindrical, and (d) degraded.

Shape also plays a crucial role in determining the hazardous effects of microplastics (Marrone et al., 2021). It is noted in the study of Almeida (2023), that the shapes of plastic microparticles play a crucial role in determining their mobility. To capture the varied settling behaviors influenced by different MP shapes, Rodríguez - Seijo and Pereira (2017), categorized them into three distinct groups: monodimensional (e. g., fibers, lines), bidimensional (e. g., films, flakes, flat thin particles), and three - dimensional (3D) (e. g., irregular fragments, pellets, ovoids, cylinders, and spherules) classes.

Notably, microplastics with fibrous or filmous characteristics exhibit limited settling through water columns, rendering them more bioavailable across all subsurface layers of aquatic systems. Conversely, three - dimensional forms display a heightened capacity to settle. Some of the plastics collected has irregular shape such as the cylindrical and angular MPs. This may suggest that these MPs are secondary MPs that originated in the long - term aquatic environment through photochemical, mechanical, and biological processes (Wang et al, 2021). It has been observed that during sample collection, plastic bottles and bags, fishing nets, and food wrappers were often observed in the surroundings of the reservoir, even found floating in the water surface. These hard plastic and outer packaging might be the source of fragments, plastic bags might be the main source of films (Nor, N. H. M. & Obbard, 2014; Thompson et al., 2004.

In their ecotoxicological assessments of microplastics across diverse coastal environments, Jung et al. (2021), observed that non - three - dimensional particles had a greater likelihood of assimilation compared to their spherical counterparts. Furthermore, these authors found that non - three - dimensional particles were more abundant in coastal waters compared to open oceanic waters. These insights underscore the intricate interplay between microplastic shapes, settling behaviors, and their ecological distribution, contributing valuable knowledge to our understanding of microplastic dynamics in aquatic environments.

Elongated fibrous MP must be examined thoroughly for their toxicity. Several studies suggest that the fibers have a particularly insidious shape for aquatic organisms, with interference both in the gut and in the gills, resulting in acute toxicity and high mortality (Gray &Weinstein, 2017; Kögel et al., 2020).

Color of the MPs in Nile *Tilapia*

Table 4 shows that various colors of MP were obtained from the GIT of Nile *tilapia*.

Table 4: Color of MPs obtained in the GIT of Nile *tilapia* cultured in Magat dam reservoir.

Color of MPs	Mean
White	3.00^{b} (1.22%)
Transparent	184.67^{a} (74.86%)
Red	18.67^{b} (7.57%)
Orange	0.67^{b} (0.27%)
Blue	24.00^{b} (9.73%)
Green	8.00^{b} (3.24%)
Black	7.67^{b} (3.11%)
ANOVA	**

*** - significant at 1% level of significance*
Means having the same letters are not significantly different

It can be seen in the table that the colors of MPs obtained include white, transparent, red, orange, green, blue, and black. Transparent MPs are significantly higher in terms of amount compared with other colors with orange showing the least amount.

This result coincides with some studies. According to a comprehensive assessment of floating plastics in seawater, research indicates that white and transparent/translucent plastics are the most prevalent colors, comprising 47% of the total. Following closely are yellow and brown plastics at 26%, with blue plastics representing 9% (Marti et al., 2020). It's important to note that this distribution doesn't necessarily suggest that most ocean plastics are white and transparent/translucent. The authors of the study excluded fiber plastics from their analysis due to potential airborne contamination, and fragments constituted 83.6% of all collected plastics.

In studies where fiber plastics were considered, predominant colors included blue, black, transparent, and white (Gago et al., 2017); black, grey, blue, and red (Suaria et al., 2020); transparent, blue, black, and red (Barrows et al., 2018); and transparent, white, blue, and red (Lu et al., 2021), respectively.

The results confirmed that these MPs are composed of polyethylene (PE), polyester (PES), polypropylene, (PP) and polyamide (PA) polymers. The results were not surprising, as these polymer types were widely found in marine and freshwater environments (Burns & Boxall, 2018; Erni - Cassola et al., 2019; Yang et al., 2021). The abundance of these polymer types in the environments could be due to improper disposal of plastic waste, as they accounted for 80% of the global plastic waste generated in 2015 (Geyer, 2017).

The PE and PP might be derived from the abrasion of fishing tools, since they are widely used in fishery activities around the world, as well as the packaging used for foods and manufactured products. PE and PP are less dense polymers that will usually float on the surface of the water and are likely to be ingested by pelagic species, while demersal species tend to ingest dense plastics such as PES and PA because they usually suspend in the water column or deposition in the seabed. PA and PES are widely used in fishery activities and the clothing industry.

The abundance of PA and PES in the environment is mostly originated from the effluent of washing clothes and the usage of fishery tools. For some studies, only part of the plastics extracted from the samples were tested with the polymer characterization test, which could lead to a potential bias of these results.

In addition, an important source of transparent and white MPs might be plastic bags, extensively used in daily life. Furthermore, this prevalence of colorless particles could also be due to the loss of color that many colored particles, especially some lines and films, undergo once they enter the surface waters. In turn, some white plastics had turned pale yellow and, not surprisingly, showed rounded corners, due to environmental exposure over a long period of time (Marrone et al., 2021).

4. Conclusion

Based on the foregoing result of the study, Nile *tilapia* (*Oreochromis niloticus*) reared in the Magat Dam Reservoir exhibit signs of ingesting MP, with fibers being the most discovered form. A sizable fraction of these MPs has an elongated morphology and a transparent appearance. The detection of MPs in the *tilapia* fish may provide insight into the MP contamination in this aquatic ecosystem.

5. Recommendations

It is recommended that additional studies be conducted to comprehensively assess the extent and sources of microplastic pollution in the Magat Dam Reservoir ecosystem. Long - term monitoring efforts can provide valuable data on trends in microplastic abundance and distribution.

Different stakeholders should develop and implement measures to reduce the input of microplastics into the reservoir. This may involve improving waste management practices, implementing policies to regulate plastic use, and raising public awareness about the environmental impacts of plastic pollution.

Furthermore, monitoring of microplastic contamination may be incorporated into existing fishery management practices. This can help assess potential risks to human health associated with consuming fish from the reservoir and inform management decisions aimed at minimizing exposure to microplastics.

In addition, educate local communities, stakeholders, and policymakers about the detrimental effects of microplastic pollution on aquatic ecosystems and human health. Promote initiatives to reduce plastic consumption, encourage recycling, and support the use of alternative materials.

Lastly, collaboration among researchers, government agencies, non - governmental organizations, and community groups may be fostered to address microplastic pollution comprehensively. Collaboration may facilitate the sharing of knowledge, resources, and expertise needed to develop effective solutions.

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