Review Article
DOI: https://doi.org/10.70749/ijbr.v3i3.785

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://induspublishers.com/IJBR
ISSN: 2960-2793/ 2960-2807

# Ecological Toxicity, Oxidative Stress and Impacts of Microplastics on Fish Gills

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## ARTICLE INFO

**Keywords**
Microplastic, Gills, Oxidative Stress, Fish, Toxicity.

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**Declaration**
**Authors' Contribution:** All authors equally contributed to the study and approved the final manuscript.*Detail is given at the end.
**Conflict of Interest:** No conflict of interest.
**Funding:** No funding received by the authors.

**Article History**
Received: 02-01-2025
Revised: 17-02-2025
Accepted: 03-03-2025

## ABSTRACT

Microplastics, which are small plastic particles less than 5 millimeters in size, originate from the degradation of larger plastic items or are intentionally manufactured for various uses. These particles have become ubiquitous in marine and freshwater environments, posing significant risks to aquatic life due to their ability to absorb and concentrate hazardous pollutants. The exposure to Microplastics (MPs), leads to DNA damage in fish that alters the hematological parameters and causes oxidative stress, thereby impacting the overall health of aquatic organisms. MPs also induce an imbalance in reactive oxygen species (ROS) production and antioxidant capacity, causing oxidative damage. In addition, MPs impact immune responses due to physical and chemical toxicity and cause neurotoxicity, altering AchE activity. This review highlights the toxic effects of MPs in fish through various indicators were examined including bioaccumulation, hematological parameters, antioxidant responses, immune responses and neurotoxicity in relation to MP exposure, facilitating the identification of biomarkers of MP toxicity following exposure of fish. This study highlights that the digestive tract contains more microplastics (MPs) than the gills, with fragments, fibers, films, and pellets being the predominant types. FTIR analysis identified polyethylene, polystyrene, polyvinylchloride, polyamide, and polycarbonate in MPs from both gills and the digestive system. MPs pollution triggered oxidative stress responses in gambusia from the East Java Brantas River. While PVC-MPs did not significantly affect gill histopathology or ion regulation, MPs combined with Cu were more toxic than individual pollutants. These findings emphasize the need for further research on the combined effects of MPs and heavy metals on aquatic ecosystems.

## INTRODUCTION

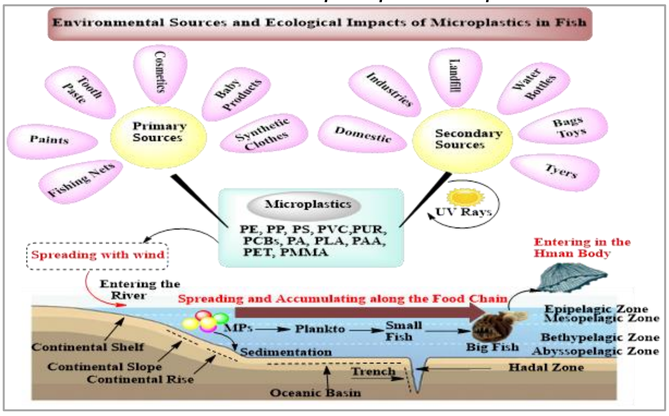
Plastics have diverse applications in several sectors such as packaging, building, autos, electronics, electrical items, textiles, agriculture and industry (Wang, Ge and Yu 2020). According to Zhang et al. (2021), the worldwide plastic production in 2018 amounted to almost 360 million tonnes, with an estimated 80,000 tonnes entering the aquatic environment. Approximately 10% of plastic waste generated worldwide equivalent to 9.5 million tonnes year, is present in the marine environment. This poses a significant threat as it is considered a hazardous substance (Iheanacho and Odo 2020). Plastics are categorised into many categories based on the material used and structure, including polyethylene (PE), polystyrene (PS), polyvinylchloride (PVC), polyethylene terephthalate (PET), polyamide (PA), polypropylene (PP) and ethylene vinyl acetate (EVA), exhibit distinct attributes and applications (Wang et al. 2020). In 2018, global plastic production reached approximately 360 million tons, of which 80,000 tons are estimated to flow into the aquatic environment (Zhang et al. 2021). Approximately 10% of plastic waste produced globally is found in the marine environment (9.5 million tons of plastic waste annually), representing a major hazardous substance(Iheanacho and Odo 2020). Plastics are divided into various types, such as poly ethylene (PE), polystyrene (PS), polyvinylchloride (PVC), polyethylene terephthalate (PET), polyamide (PA), polypropylene (PP) and ethylene vinyl acetate (EVA) depending on the material used and structure and have different characteristics and usage.

Plastics contain chemicals from various plastic additives, such as plasticizers (phthalates, bisphenol A), colorants, ultraviolet (UV) filters and flame retardants in the manufacturing process. Furthermore, organic contaminants like polychlorinated biphenyls (PCBs), organochlorine pesticides, polycyclic aromatic hydrocarbons (PAHs) and metals can be adsorbed on the surface of MPs when moving in the aquatic environment (Koelmans et al. 2016, Rochman et al. 2015). MPs can enter the food web through accidental consumption by organisms that misidentify them as food, because of their small size, leading to cumulative impacts on predators higher up the food chain through bio-magnification (Au et al. 2017). Consequently, MPs impact zooplankton, mollusks, crustaceans, and all life stages of fish, marine turtles, aquatic mammals and seabirds (Pannetier et al. 2020). Since fish are top predators and representative groups of aquatic ecosystems, they are considered to be the most important biomarkers for evaluating MP toxicity (Jambeck et al. 2015). Thus, MP consumption impacts the aquatic ecosystem but also threatens food safety, because fish are an important protein source for humans.

Microplastics (MPs) are synthetic polymeric matrix components that are small, solid and water-insoluble particles, which break down from plastics in aquatic environments and directly impact aquatic animals (Frias and Nash 2019). The mean size of MPs is generally <5mm, but recent studies suggest MPs should be classed as ranging from 1 to 1000 µm (Parker et al., 2020). Evidence of freshwater and marine fish consuming MPs has been widely documented. MP consumption has physical and chemical toxic effects, including mechanical injury (Wright and Kelly 2017). For instance, the consumption and accumulation of MPs in the digestive tract causes physical blockages and inflammatory reactions in aquatic organisms. Such blockages cause internal damage, including intestinal perforation and ulcerative lesions and potentially gastric rupture and deformation, leading to death (Law 2017). Other adverse effects include energy disturbance, decreased reproduction growth, oxidative injury, metabolic disorders, cellular lesions, endocrine disruption, decreased immunity, neurotransmission disorder and genotoxicity, also potentially leading to mortality (Ding et al. 2018, Lu et al. 2016).

**Figure 1**

*Potential sources and transfer of MPs in fish*




Microplastics (MPs) are microscopic (1–1000µm) pollutants and due to their ubiquitous occurrence, persistence and adverse effects on biota, they are emerging as a top concern worldwide. MPs originate from primary or secondary sources (Wright, Thompson and Galloway 2013). Primary sources include manufactured microbeads and microfibers used in various industries such as textiles, skin products and cleaning agents, while secondary sources are from the degradation of larger plastics into smaller particles. The most commonly found plastics in the environment include polyethylene, polypropylene and polyvinyl chloride (Burns and Boxall 2018). The principal environmental risk associated with MPs is their bioavailability to a large variety of aquatic organisms, including fish(Li et al. 2016, Wright et al. 2013).Oxidative stress occurs when there is an insufficient ability of the organism to neutralize excessive reactive oxygen species (ROS) from their own suite of antioxidant enzymes. Antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), Glutathione reductase (GR), Glutathione S-transferease (GST). The origin of ROS can be from within the animal and/or from the environment and an accumulation of ROS accumulation leads to substantial damage to lipid membranes, DNA and proteins (Kelley, Schatz and Salzberg 2010, Livingstone 2001). Among the enzymes, SOD converts ROS to hydrogen peroxide (H2O2), while CAT and GPX catalyze the reduction of H2O2 into water and oxygen. Meanwhile, GR and GST are involved in resisting oxidative stress and detoxification, which are crucial mechanisms to withstand the toxic effects of pollutants. The cause for oxidative stress in MP-exposed fish may result from the physical presence of MPs causing tissue damage. A sign of lipids becoming oxidized and thus damaged includes accumulation of malondialdehyde (MDA) and H2O2. A more general stress response is an increase in cortisol and their receptors, in which cortisol is a well-known immunostimulant and inhibitor to growth hormones (Madison et al. 2015).

It has been shown that the exposure of nanoplastics increased cortisol levels in zebrafish (Brun et al. 2019), but there is limited information on the effects of MPs to stress responses in fish. Furthermore, it is well acknowledged that the physio-biological and cellular processes are regulated at the gene level. Hence, this work also aims to extend our understanding on how environmental pollutants such as MPs modulate the expression of various key genes regulating stress response and growth performance. Exposure to MPs can also induce adverse histo-pathological changes in the fish, which has included inflammation in the liver and intestines (Qiao et al. 2019), hepatic adenoma (Rochman et al. 2015), necrosis and hemorrhaging as well as substantial gills damage. The cause for such damage to the gills and intestine could be from the physical abrasions of the MPs themselves (Karami et al. 2016). Gills are the main site for the gaseous and ionic exchange and are directly exposed to waterborne contaminants including MPs, which can permeate (via passive diffusion) across the gill membrane and transport through the circulatory system to the liver, brain and other organs (Barboza et al. 2020, Barboza et al. 2018a).

The majority of ion transport in the gills of freshwater teleosts is regulated by energy-dependent basolaterally located Na+/K+-ATPase enzyme that generates the driving force for the uptake of Na+ from the water. Along with Na+/K+-ATPase, the electro-chemical gradient for Na+ uptake via gill is also facilitates by apically located H+-ATPase in as sociation with the (apical) Na-channel (Lin and Randall 1991). Several environmental anomalies have been shown to modify the activities of these ion-transporters in fish gills (Sinha et al. 2016, Sinha et al. 2013). Gills are important respiratory organs of shrimp, which have functions such as osmoregulation and nitrogen excretion and are also involved in the immune response to remove the pathogens (Xu, Kraft and Xu 2016). The gills of shrimp directly contact with the water environment, so they are easily affected by environmental stress, resulting in physiological disorders (Duan et al. 2018b, Duan et al. 2018a).Therefore, in this study, we evaluated the effects of individual and combined stress of nitrite and microplastics on physiological function in the gills of L. vannamei.

**Preparation of Microplastics and Diets**

Microplastics, defined as microscopic plastic particles ranging from 0.1 µm to 5 mm in size, are widespread anthropogenic pollutants that impact terrestrial, freshwater, and marine ecosystems worldwide (Cózar et al. 2014, Eriksen et al. 2014).The cryotome protocol was optimized to produce microscopic particles of small size from polypropylene (PP) origin plastic fibers with diameter of 0.9 mm (Cole 2016). PP microplastic particles were obtained by cutting pieces of approximately 1.0–0.9 mm in length from plastic fibers. Particle size, surface structure and shape are important properties of microplastic particles. For this purpose, the characterization of microplastic particles was performed using energy dispersive X-ray spectroscopy (EDX) and Scanning Electron Microscopy (SEM) (Microscope). The microplastic particle size was performed with Image J software (Ver. 1.53). In the study, additive-free diets containing only basic feed ingredients (55% crude protein, 20% crude oil, 12% crude ash, 11% vitamins and 2% crude fiber) and additive diets containing PP microplastics (100 mg/g and 250 mg/g) were prepared.

**Microplastic Bioconcentration in Gills**

The rising prevalence of microplastics in oceans is a growing concern for marine ecosystems. These tiny particles enter marine organisms through ingestion or directly via body surfaces like gills and skin (Ikuta et al. 2022). Plastic with particles size <5 mm in the diameter are called microplastics (Andrady 2011, Wayman and Niemann 2021). Microplastics are small enough to fall within the size range of food particles typically consumed by many aquatic animals (Au et al. 2017). The quantity of microplastics in oceans globally is on the rise, prompting concerns about their effects on marine ecosystems and human health (Thushari and Senevirathna 2020). Microplastics pose various physical risks to marine organisms, including gastrointestinal blockages, inflammation, and structural damage. They can also lead to reduced feeding, hormonal imbalances, nutritional deficiencies, reproductive failures, and alterations in metabolic profiles (Kolandhasamy et al. 2018, Amelia et al. 2021). Microplastics can be taken up by gill cells through phagocytosis, as demonstrated by research on deep-sea mussels, which revealed that specific gill cell types engulf these particles directly (Ikuta et al. 2022).

The bioaccumulation of microplastics in marine organisms, including fish, occurs via the food chain, with these particles entering the fish through both their gills and mouths. Internal contamination by microplastics can lead to various effects, including heightened toxicity and growth inhibition (Nabila and Patria 2021). The presence of microplastics in the water elevated mercury concentrations in the gills and liver of juvenile *Dicentrarchus labrax*. Both microplastics and mercury, whether individually or in combination, induced oxidative stress in these organs (Barboza et al. 2018b). Following ingestion or passage through the gills, microplastics can be absorbed and distributed throughout the circulatory system. As a result, these particles may become incorporated into various tissues and cells (Barboza et al. 2018a). The highest levels of microplastics resulted in oxidative damage to lipids in the gills. In fish exposed to mixtures of microplastics and mercury, toxicological interactions were observed, indicating an additive effect in gills and either additive or synergistic effects in the liver at low concentrations.

These findings highlight the necessity for further research into how microplastics influence the bioconcentration, bioaccumulation, and toxicity of other environmental contaminants across various species (Barboza et al. 2018b).

The consumption of microplastics (MPs) may lead to both anatomical and functional alterations in the digestive tracts of fish, potentially resulting in dietary and developmental problems (Huang et al. 2022, Jabeen et al. 2018). The majority of studies have focused on Danio rerio, revealing that microplastics (MPs) commonly lead to oxidative stress, reduced mobility, disruptions in gene expression, and damage to reproductive organs (Mu et al. 2022, Zhao et al. 2021). Additionally, the presence of the particles in the gills may have decreased the oxygen uptake leading to hypoxia, subsequent reduction of the aerobic cellular energy production, as hypothesized for Daphnia magna exposed to the same type of microplastics. (Barboza et al. 2018a). The buildup of microplastics in the gills acts as a marker for environmental contamination, providing a reflection of real-time exposure levels within aquatic ecosystems (Yin et al. 2022).

**Effects of Bioaccumulation of MPs in Fish Gills**

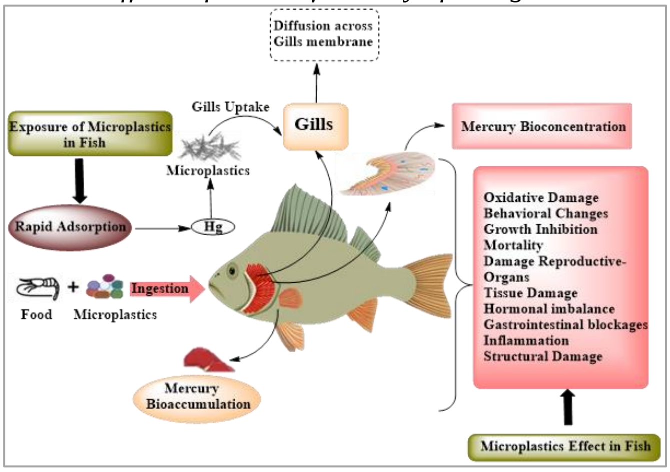
Microplastics are often accumulated in the gills of aquatic animals (Watts et al. 2014). If fish uptake microplastics through their gills, which are adsorbed with mercury, this could lead to higher mercury concentrations in the gills. Additionally, the release of mercury from these particles may result in increased accumulation in other organs, such as the liver (Turner and Holmes 2015). Physiologically, chloride cells in the gills of freshwater fish actively absorb ions in the water and discharge large amounts of water via urine to prevent ion-loss in low osmotic pressure freshwater environments. In contrast, marine fish take in large quantities of water to prevent dehydration in high osmotic pressure marine environments. Therefore, these physiological differences lead to higher accumulation in marine fish when exposed to the same concentration of MPs (Lee et al. 2019, Assas et al. 2020). MP accumulation is also influenced by a variety of factors, including fish species, exposure concentration and time, particle size and food intake (Meng et al. 2018). In particular, the size of MP particles is a major factor in determining accumulation in fish. For instance, MP size determines the degree to which they are transported in the circulatory system, driving differences in tissue accumulation (Lu et al. 2016). MPs of <5μm can pass through intestinal cells via transformation, enter the circulatory system and move to tissues. Particles of 5–150 µm size pass through the intestinal membrane through absorption and are reorganized in the circulatory system (Jovanović et al. 2018).

The bioaccumulation profile in fish exposed to MPs based on exposure pathways (freshwater versus sea water, waterborne exposure and dietary exposure). Intestinal damage caused by exposure to MP can lead to villi cracking and intestinal cell division and it altered the level of ions such as calcium in the intestine, resulting in functional impairment (Lei et al. 2018). In addition, the absorption pathway shows that both ingestion-intestinal-blood-tissues and waterborne particles can be distributed to water-gills-blood-tissues orally. The authors suggested that the 0.1mm sized PS-MP can even reach brain tissue through circulating blood. The size of MPs is closely related to accumulation. For instance, smaller MPs tend to remain for longer durations in tissues, while larger particles promote the equilibrium of bioaccumulation in fish (Rist, Baun and Hartmann 2017). Gills are a major tissue in the respiratory process that control ions and acid bases and are directly exposed to a variety of toxic substances in the aquatic environment through contact with water. Consequently, gills provide a major route for the introduction of various pollutants to fish tissue (Roda et al. 2020). The route of exposure (water-borne exposure and food exposure) is the most important factor in determining accumulation following exposure to toxic substances but in MP exposure, particle size was more decisive in determining the tendency of accumulation than exposure route. In addition, the MP accumulation in the brain can act as a direct toxicity to the central nervous system, because it can reach the brain by penetrating brain-blood barrier depending on the size of the particles. In addition, the tendency of accumulation is different depending on the size of the MP particles but the accumulation of each tissue acts as toxicity and causes damage to the tissues so that they cannot function properly. The role of intestinal tissue in digestion and absorption means that it comes into direct contact with contaminated substances through feeding activity, leading to high MP and toxicant accumulation (Kim and Kang 2017). The role of the liver and kidneys in the detoxification and excretion of particles from circulating blood exposes them to toxic substances, potentially leading to high MP accumulation (Kim and Kang 2015).

The accumulation of MPs in the gills and intestines of fish following exposure indicates that respiration and ingestion are the major absorption pathways for MPs. In addition, large quantities of MPs can accumulate in the gills due to their large surface area and water-borne exposure. MPs in the fish body can be absorbed across cells and intestinal barriers and remain toxic in tissues for a significant amount of time. Yet, MPs ingested in the digestive tract can sink to the bottom of the aquatic environment through excretion, posing a potential risk to benthic organisms (Yin et al. 2018). Determining the tendency of MPs to accumulate in fish following exposure provides important information for identifying organs that should be targeted to infer MP toxicity and confirm MP concentration when sampling fish in the field. Various studies have demonstrated that the tendency for MPs to accumulate depends on MP size, not exposure route. (Zitouni et al. 2021) also stated that, regardless of the absorption pathway, MP accumulation largely depends on size, with accumulation being easier for smaller-sized MPs. In contrast, (Jovanović et al. 2018) argued that small MP particles can be easily removed from the liver through the circulatory system, whereas larger MP particles are more likely to remain. It is judged that the accumulation of MP and their toxic effects are more affected by the size of MP particles rather than the MP types or absorption pathways. Therefore, it is necessary to identify the target organ through various additional studies on the tendency of MPs to accumulate in fish in relation to MP size.(Kim, Yu and Choi 2021).

**Figure 2**

*Possible effects of MPs in fish body after ingestion.*




## Pathophysiological Pathways of Oxidative Stress Triggered by Microplastics in Fish

In fish, oxidative stress can arise from a variety of factors, including exposure to pollutants, infections by pathogens, and environmental stressors (Qi et al. 2020). Excessive production of reactive oxygen species (ROS) or weakened antioxidant defenses can lead to oxidative stress, which is associated with several health issues in fish, such as tissue damage, organ dysfunction, and compromised immune function (Bojarski et al. 2022). The specific mechanisms through which microplastics induce oxidative stress may involve factors such as the type, size, and shape of the plastic, as well as variations in antioxidant levels based on the fish's species and life stage (Subaramaniyam et al. 2023, Reichert et al. 2019). The research indicated that microplastics were found in the dorsal muscle, gastrointestinal tract, and gills of 49% of the fish analyzed. Additionally, fish contaminated with microplastics exhibited increased lipid peroxidation levels in the brain, dorsal muscle, and gills, along with elevated brain AChE activity (Subaramaniyam et al. 2023).

Microplastic exposure can activate toxicity pathways like oxidative stress and inflammation, potentially affecting localized areas or entering the bloodstream to impact various organs and tissues, while also bioaccumulating in the human body and causing toxicological effects (Ferrante et al. 2022). The primary constituents of microplastics (MPs) are polymers, such as polyethylene (PE), polypropylene (PP), poly (ethylene terephthalate) (PET), and polystyrene (PS). These particles vary in size, shape, and color (Kadac-Czapska et al. 2023). Microplastics can originate from two sources: primary microplastics, which are intentionally manufactured to be microscopic in size, and secondary microplastics, which result from the breakdown of larger plastic items (Wright et al. 2013). The toxicity associated with microplastics (MPs) is significantly heightened when combined with additives such as bisphenols, phthalates, and persistent organic pollutants (POPs), compared to the toxicity caused by MPs alone (Chen et al. 2024, Deng et al. 2021, Rubin and Zucker 2022).

## Effects of Microplastics-Induced Oxidative Stress on Cells, Cell Membrane, Lysosomes, Mitochondria, ER, Tissue, Organs and Organisms

Microplastics (MPs) impact specific cellular organelles, and when they interact with the cell membrane, they cause physical damage to the cells (Liu et al. 2021). Signs of lysosomal damage include decreased lysosomal hydrolase activity, alterations in lysosomal pH, and impaired autophagy (Deng et al. 2022). These organelles may be affected by microplastics (MPs) either directly or indirectly (Fiorentino et al. 2015). Morphological manifestations of microplastics (MP) damage include mitochondrial swelling, weakened myelin in the mitochondrial membrane, loss or reduction of cristae, decreased enzyme activity, altered membrane permeability, and damage to mitochondrial DNA (Wei et al. 2021, Zhang et al. 2022b).

**Table 1**

*Effect of different lethal doses of Microplastics on fish organs.*

| Microplastic Type | Microplastics Size | Dose | Toxic Effects | References |
|---|---|---|---|---|
| Propylene Ethylene | 50–200 µm | 2mg/L, 3mg/L | Gills have a higher concentration of microplastics, while oxidative damage primarily impacts the liver and gills | (Köktürk et al. 2024) |
| Polystyrene | 0.5 µm, 15 µm | 100 µg/L, 500 µg/L | Induced oxidative stress and liver congestion have led to changes in gut | (Hao et al. 2023) |

| | | | | |
|---|---|---|---|---|
| | | | microbiota and significant intestinal damage. | |
| Polyacrylamide | 0.1–0.4 mm | 0.018, 0.03 0.09 g/L | Decreased levels of CAT and GSH have been observed, alongside elevated levels of MDA and lipid peroxidase | (Raza et al. 2023) |
| Polypropylene | 8–10 µm | 1 mg/g and 10 mg/g | Induce Oxidative Stress | (Bobori et al. 2022b) |
| Polytetrafluorethylene | 31.7 µm | 1000 µg/mL | ROS decrease, Increase secretion of IL-6 | (KC et al. 2023) |
| Polystyrene | 8.9 µm and 1.14 µm | 10 and 100 µg/mL | ↓intracellular H2O2 levels | (Saenen et al. 2023) |
| Polyvinylchloride | 0.16, 1.82 µm | 24, 48, and 96 µg/mL | ↑ROS, ↑ activity of GSSG, ↓GSH and Mitochondrial membrane potential collapse | (Salimi et al. 2022) |
| Polystyrene | 20 µm | 1 mg/L for 21 days | ↑ Activity of SOD, ↓ Activity of CAT, GPx, and GST | (Ribeiro et al. 2017) |
| Ethylene Propylene copolymer | <200 µm | 0.1 and 1 mg/L for 21 days | ↑ Activity of CAT and SOD, ↓ activity of GPx and GST, ↓ Mitochondrial membrane potential | (Félix, Carreira and Peixoto 2023) |
| Polystyrene | 1 µm | 1 mg/L for 3days | Induce Oxidative stress, ↑ expression of GST-4 | (Hu and Palić 2020) |
| Carboxylate-modified PS | 0.3 µm | 1 mg/L for 2 days | ↑SOD, ↓ GSH, ↑ MDA, ↓AChE | (Siddiqui et al. 2023) |
| Polystyrene | 5–5.9 µm | 0.01, 0.1, 1, and 100 mg/d for 42 days | Activate p38 MAPK, level of Casp-3, TNF-α, IL-1β, and IL-6 in the testicular tissue and ↓ LDH and SDH | (Jeon et al. 2023) |

Microplastic particles in gambusia gills and digestive tract were found at all sampling sites. Microplastic particles were discovered in the gills and digestive tract at all sampling sites. MP abundance is minimal because microplastics can stick to the gill surface and be rinsed away by water or other environmental causes (Su et al. 2019). The microplastics found in the gills result from their retention in this organ during water filtration depending on the type, size of the microplastic and the efficiency of the filtering apparatus (Barboza et al. 2020). Microplastics did not permanently accumulate in high numbers in the gills of adult zebrafish after 6 or 24h of incubation because most of the particles simply adhered to the mucus layer on the filaments, which were continuously excreted (Batel et al. 2018). Microplastics stuck in the gills can reduce respiratory efficiency can cause hypoxia, can cause physical damage to the gills, such as filament fractures and facilitate the entry of microplastics and other particles that increase the likelihood of infection (Jabeen et al. 2018, Movahedinia, Abtahi and Bahmani 2012). Microplastics, on the other hand are more difficult for fish to digest than food which is why MP remains in the intestines (Hastuti, Lumbanbatu and Wardiatno 2019). Although the gastrointestinal system is the most prevalent route for microplastic absorption, the gill is also a significant pathway for water-borne MP uptake that causes short-term toxicity in fish (Sun et al. 2019).

Four types of MPs were found in the gills and digestive tract as a fragment, fiber, film and pellet In several studies, the same type of MP was identified in organisms (Jabeen et al. 2017, Lusher, Mchugh and Thompson 2013, Ory et al. 2018). The most abundant type of MP in the gills and digestive tract is fragments followed by fiber, films and pellets. The percentage of fragments in the gills is 43.7% and in the digestive tract is 48%. During the study, plastic bags and bottles were abundant in the watershed, which may have contributed to the highest number of fragments compared to the other types. The abundance of fragments is due to the fact that the majority of the debris on the river's banks is plastic bottles and other domestic plastic waste, which is a source of fragments (Layn and Emiyarti 2020). Plastic waste such as packaging materials, plastic bags, containers, pipe pieces and other plastic objects that decompose in natural water bodies are the source of this type of fragment (Ariyunita, Dhokhikah and Subchan 2021, Eerkes-Medrano and Thompson 2018).

The MPs size distribution in this study ranged from 0.0001 mm–1.0 mm. The most prevalent MPs size was 0.05 mm–0.1 mm which was 40.7% of the total MPs in the gills and 40% of the total MP in the digestive tract. Because small MPs sizes are easier to digest, they can enter internal organs and increase the bioavailability of related chemicals (Eerkes-Medrano and Thompson 2018). The FTIR results on microplastic samples from the gills and digestive tract revealed five different kinds of polymers, including polyethylene (PE), polystyrene (PS), polyvinylchloride (PVC), polyamide (PA) as nylon and polycarbonate (PC). MPs with fragment type is composed of several types of polymers such as PE (Scherer et al. 2018), PS (Chércoles Asensio et al. 2009, Ding et al. 2020a), PVC (Ding et al. 2020b). Fiber can be formed from PE polymers (Ding et al. 2020b) and PA (Tanaka and Takada 2016). Films can be composed of any type of PE polymer (Laurén and McDonald 1987). While the pellet or microbeads type is composed of PC polymer (Tanaka and Takada 2016) and PS (Scherer et al. 2018) . PVC is a very important plastic polymer in terms of its negative effects on environmental ecology and possible hazardous effects on organisms. Because it contains significant chemical additions such as phthalates, which can bind to molecular targets in the body and affect hormones (Facchetti et al. 2020).

**Effects of Microplastics on Fish Physiology, Behavior, Reproduction, and Growth or Development:**

MPs can be located in various layers of water, including surface water, the water column, and the sediment at the bottom. The distribution of microplastics is influenced by their characteristics (type, size and polarity), surface biofilms, and water flow conditions. These factors determine their bioavailability and toxicity to aquatic organisms (Wang et al. 2019). Microplastics (MPs) can induce inflammation in fish, elevating inflammatory markers like C-reactive protein and cytokines, while also disrupting lipid metabolism, leading to increased cholesterol and triglyceride levels. Additionally, MPs can interfere with the endocrine system, affecting hormone levels such as thyroid hormones and altering various blood biochemical parameters (Banaee et al. 2019). Ingesting microplastics can lead to altered behavior in fish, such as reduced feeding and increased risky behaviors, which may heighten their vulnerability to predation and negatively impact population dynamics (Liang et al. 2023, Yin et al. 2018).Fish frequently confuse microplastics with actual food, resulting in decreased nutrient absorption and slower growth rates (Cattaneo et al. 2023). Microplastics can disrupt the natural reproductive behaviors of fish (McCormick et al. 2020). Microplastics serve as carriers of toxic chemicals, negatively impacting fish health and fitness, and impairing their ability to choose appropriate habitats and successfully undertake migratory journeys (Lu et al. 2021).

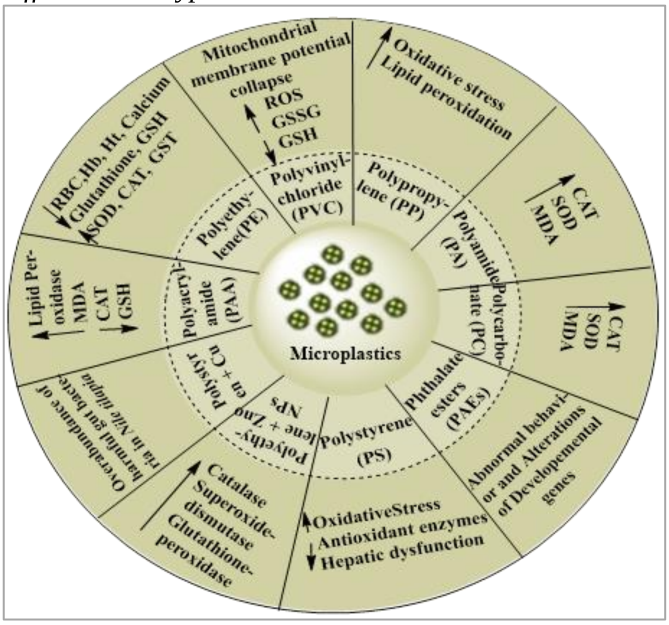
**Table 2**

*Microplastics have induced toxicity in different fish species.*

| Microplastics Type | Species Name | Toxic Effect and its Mechanism | References |
|---|---|---|---|
| PVC | *Spaurus aurata* | Impact on innate immune factors, including phagocytic activity and respiratory burst, as well as peroxidase activity in leukocytes of the head kidney | (Espinosa, Cuesta and Esteban 2017) |
| LDPE | *Spaurus aurata* | Changes in antioxidant enzyme activities, lipid peroxidation, and behavioral alterations (such as social interactions and feeding behavior) | (Rios-Fuster et al. 2021) |
| PAEs | *Danio rerio* | Abnormal behavior and alterations of the developemental genes | (Qian et al. 2020) |
| PS + Cu | *Oreochromis niloticus* | Overabundance of harmful gut bacteria in *Nile tilapia* is associated with a simultaneous decline in the fish's immune response | (Zhang et al. 2022a) |
| PE + ZnO-NPs | *Gambusia holbrooki* | ↑ Catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase activity due to oxidative stress induced by ZnO-NPs, both alone and in combination with PEMPs | (Banaee et al. 2023) |
| PS | *Nothobranchius guentheri* | ↑oxidative stress, ↓ antioxidant and digestive enzymes, and the hepatic dysfunction | (Xia et al. 2020) |
| PE | *Pseudobagrus fulvidraco* | ↓RBC, Hb, Ht, calcium, total protein, and magnesium, ↑in the activity of antioxidant enzymes SOD, CAT, GST, ↓ glutathione (GSH) were decreased. | (Lee, Kang and Kim 2023) |
| PVC | *Cyprinus carpio* | ↑ oxidative stress and ↓ MDA and the antioxidant activity | (Xia et al. 2020) |
| PA, PC | *Gambusia affinis* | ↑CAT, SOD and MDA | (Buwono, Risjani and Soegianto 2022) |
| PP | *Danio rerio* , *Perca fluviatilis* | ↑ Oxidative stress and Lipid peroxidation | (Bobori et al. 2022a) |

**Figure 3**

*illustrates the Oxidative stress parameters induced by different MPs types.*




**Exploring the Ecological Impact of Microplastics on Aquatic Food Chains**

Ingesting microplastics can disrupt fish behavior, physiology, and survival, leading to significant ecological consequences for aquatic ecosystems, including species abundance and overall ecosystem health (Aslam et al. 2023). Rising microplastic pollution can disrupt aquatic ecosystems by altering the food web, biodiversity, habitat, nutrient cycles, and weakening the overall health and stability of ecological communities (Dantas et al. 2024, Galli et al. 2022). Herbivorous fish, mainly consuming plant material, may face lower microplastic exposure, but could still ingest them if microplastics are attached to algae or other plants (Pan et al. 2021). Carnivorous fish and apex predators may face higher microplastic exposure due to biomagnification, accumulating greater levels of these particles as they consume contaminated prey (Garcia-Garin et al. 2019). Fish species with more selective feeding habits, focusing on specific prey, may experience different levels of microplastic exposure (Filgueiras et al. 2020). The bioaccumulation of microplastics in migratory fish species can disrupt food webs, nutrient cycles, and biodiversity, ultimately weakening ecosystem resilience and highlighting the need to address microplastic pollution for ecosystem health and species preservation (Banaee et al. 2024). Recent research on the occurrence, accumulation, environmental impacts, and detection methods of plastics in freshwater has increased, highlighting growing attention on the presence of microplastics in these ecosystems (Li, Liu and Chen 2018). In 2005, the United Nations Environment Program (UNEP) estimated that ships alone were responsible for dumping up to 5 million tons of plastic waste into the oceans worldwide (Löhr et al. 2017). The growth of aquaculture has led to increased use of plastic fishing nets, which, when discarded improperly, contribute to ocean pollution, with U.S. statistics estimating 30 million pounds of plastic fishing gear entering the ocean annually (Lynch 2018).

Microplastics have been found at both lower and higher trophic levels in marine environments, contaminating zooplankton, copepods, fish, invertebrates, seabirds, and mammals (Hollman, Bouwmeester and Peters 2013). Microplastics enter the human food chain through contaminated foods and may impact human health, with inhalation also serving as a potential route of exposure(Karbalaei et al. 2018). The ingestion of microplastics can disrupt energy balance, interfere with metabolic processes, and impair the immune system (Prata et al. 2020). Microplastics on the water surface or suspended in the water column can reduce light penetration and increase turbidity, affecting the growth of phytoplankton and aquatic plants. They also degrade water quality by introducing toxic substances, promoting harmful algal blooms, depleting oxygen levels, and releasing toxins that harm aquatic life (Jayapala et al. 2024). Microplastics in fish habitats can disrupt predator-prey interactions by impairing fish's sensory abilities, making them more vulnerable to predation. Exposure to MPs can also weaken fish, reducing their agility and ability to escape predators. These interconnected changes can trigger cascading effects across aquatic ecosystems. Therefore, it is crucial to implement solutions such as reducing plastic production, improving recycling, using biodegradable materials, and enhancing waste management to minimize plastic waste and its environmental impact (Banaee et al. 2024).

## CONCLUSION

In conclusion, the digestive tract is more contaminated with MP than the gills. The types of MPs found in the gills and digestive tract are fragments, fiber, films and pellets. FTIR measurements identified five types of polymers, including polyethylene (PE), polystyrene (PS), polyvinylchloride (PVC), polyamide (PA) and polycarbonate (PC) in microplastic samples from the gills and digestive system. Due to MPs pollution, SOD, CAT and MDA reactions were observed in gambusia gills and digestive tract of the East Java Brantas River. This study demonstrates that MPs abundance has both direct and indirect impacts on oxidative damage. The results also illustrate that neither of the tested PVC-MPs concentrations had a significant adverse impact on gill histopathology, ion-regulation function of gills or on plasma ion homeostasis. In most of the evaluated biomarkers, the combination of MPs with Cu was revealed to be more toxic than the individual pollutants, evidencing that the interaction of plastics with heavy metals can cause more harm to aquatic organisms. The present study contributes with new insights into the molecular and cellular mechanisms involved in MPs and Cu long-term toxicity in fish gills. However, more comprehensive studies are still needed to increase our knowledge about the combined effects of MPs and absorbed pollutants and the potential consequences to aquatic ecosystems.

**Author Contribution**

NW and AN: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing—original draft, Writing - review and editing: FS, MA, SI, SA, MSA: Data curation, Formal analysis, Investigation, AM, SMJRR, ZB and BT Writing—review and editing.

### Availability of Data and Material

All the data is available and can be obtained on reasonable request from the corresponding author .

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