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MICROPLASTICS CONTAMINATION IN FRESHWATER FISH – A REVIEW

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ABSTRACT

The excessive use of microplastics (MPs) has raised global concerns due to their detrimental effects on aquatic life. The present review has comprehensively covered the current knowledge of MP contamination, their uptake, distribution, impact on organs and biological functions, bioaccumulation, and biomagnification in higher trophic levels. MPs originate from various sources and can accumulate in freshwater fish, which are an important source of food for human beings. Numerous fishes ingest MPs through their mouth, skin, and gills but the primary route is through the gut. Once in the gut, MPs can persist for a long time and then distribute to the entire body via the circulatory system, accumulating in the tissues. The ingestion patterns of MPs are often related to environmental factors (e.g. habitat features, local urbanization) and individual traits (e.g. trophic level, body size). Understanding the interactions between MPs and other environmental stressors is crucial in addressing the increasing plastic pollution and its impact on freshwater ecosystems. The quality of these ingestion studies is questionable due to the lack of standard methods for sampling, extraction, and identification of MPs. New techniques for measuring MPs in freshwater ecosystems and improved waste management are essential to mitigate this issue.

Keywords: Microplastic, Freshwater, Fish, Contamination, Microscopic particles.

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1. INTRODUCTION

Plastic pollution is a common and major environmental problem of the 21st century (Raju et al. 2023). Plastics are designed through polymerization and condensation reactions. They are elastic, low-cost, lightweight, strong, water-resistant and also act as insulators. They are not biodegradable, but some are biodegradable and can be decomposed by hydrolysis, by the action of microbes, or in the occurrence of ultraviolet (UV) light (Bhardwaj 2022). In the 1970s, for the first time MPs were found in marine environments. These tiny particles were added to marine via lakes and rivers. The spread and effect of MPs on the aquatic environment was studied in 2004. In 2013, MPs were found in freshwater for the first time (Eriksen et al. 2013). Production of plastics is increasing day by day and the use, disposal, and recycling culture is also developing. The production of plastics increased from 1.5 million to 322 million between 1950 and 2015 due to its beneficial uses in different fields. China is producing more MPs about 30% of the world. As production is growing the process of recycling is not increasing at the same rate. Improper disposal causes the accumulation of plastics in natural habitats, mainly the aquatic environment.

MPs are greatly diverse and differ in shape, size, polymer type, color, and their component elements that all affect how they act in the ecosystem such as their transportation, adsorption capacity, degradation, and eventual destiny. Miroplastics are of two categories: Primary MPs and secondary MPs (Fig. 1). Primary MPs are small, microscopic plastic grains that are intentionally synthesized and commonly used in cosmetic products like facial cleansers and secondary MPs are the tiny particles that are the remains of deteriorated plastic waste (González-Pleiter et al. 2019). Plastics that are present in the upper water column become more fragile to decompose while plastics that are present in the deep sediment there for decades. Environmental pressure like UV rays, temperature, and mechanical damage can cause fragmentation. Most MPs are transferred from land to water ecosystems. Some recreational activities like boating cause direct addition of MPs and waste into lakes and rivers (Roychand and Pramanik 2017). The textile industry uses synthetic polyesters to make clothes. MPs are secondarily formed as these clothes are washed or worn out (Belzagui et al. 2019). The sludge from wastewater treatment plants contains 98% MPs. This sludge can be used as fertilizer if not disposed of to the ground (Rolsky et al. 2020). Plastic particles entered into aquatic ecosystems and their floating, sinking or settling capability depend on the density or shape or type of particle and also on environmental features (water density, salinity, flow rate etc.) and aquatic processes (e.g. water currents and storm events) (Zhang et al. 2020). The ultimate fate of MPs is to accumulate in sinks, such as river sediments (de Villiers 2019). Various sources of MPs in the aquatic environment are depicted in Fig. 2.

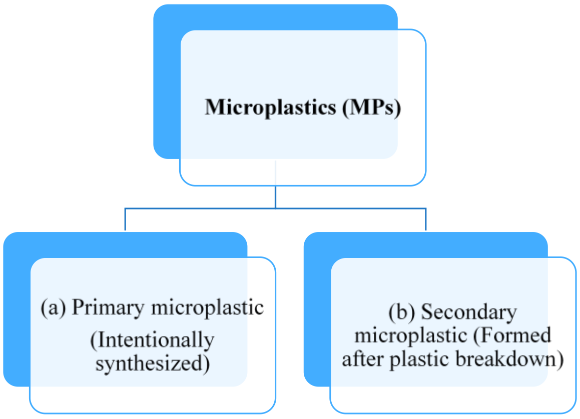

Fig. 1: Types of MPs in aquatic ecosystem (a) Primary microplastic (b) Secondary microplastic (Witczak et al. 2024).

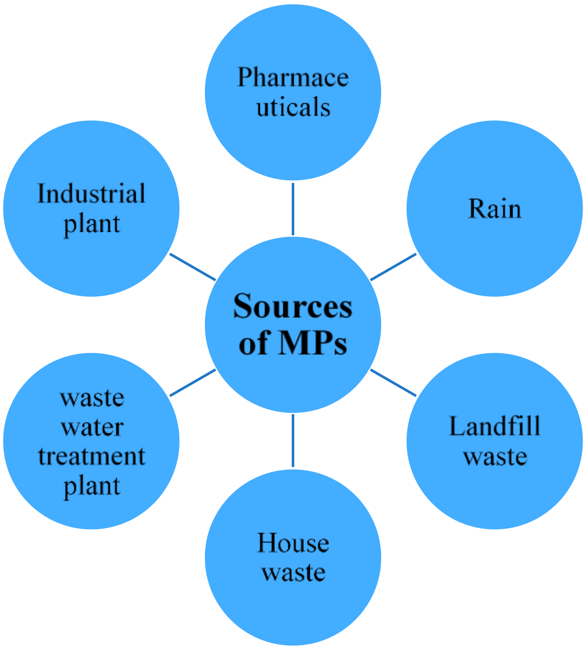

Fig. 2: Different sources of MPs in aquatic ecosystem. MPs come from several sources from industries, household plastic waste, washing clothes cosmetics, drugs, or even from wastewater plants. Other than that MPs also accumulate in the sediments of water bodies and after some time leach out from sediments and enter into the fish, thus altering the normal functioning of fishes and aquatic life (Ghosh et al. 2023; Samal et al. 2024).

The low-density polymers (polyethylene) float on the water surface while higher-density particles (polyvinyl chloride) sink at the bottom where benthic organisms are more likely to get in contact (Ockenden et al. 2021). The bioavailability of MPs increases with a decrease in their size and is available even at lower trophic levels (Pinheiro et al. 2017). MPs have become a global problem as it is made and released globally (Alimba and Faggia 2019). Data on ingestion expose that ecological MPs are consumed by a variety of various taxas across fluctuating trophic loci, types of feeding and habitats (Gouin 2020). These MPs are found in many fish which are used as food (James et al. 2020). MPs cause many problems for the fishes by changing the function of organs and eventually death (Mallik et al. 2021). MPs affect the digestive system and produce pseudofeces, predatory behavior as it cannot recognize the original prey which can cause a deficiency of food in the body. MPs begin to store in the body organs like in the gills, guts, and stomach (Guven et al. 2017). The accumulation of MPs in the body of fish causes oxidative stress, hormonal problems, metabolic disturbance, genotoxicity, immunotoxicity, neurotoxicity and behavioral toxicity (Guven et al. 2017; Choi et al. 2018). Our study focused on uptake, distribution of MPs and their physiological, Biological and behavioral effects on fish.

2. UPTAKE AND DISTRIBUTION OF MICROPLASTICS IN FRESHWATER FISHES

In fishes, several uptake routes of MPs have been suggested (Fig. 3). Primarily uptake occurred through Active and Passive ways. In active uptake, fish can uptake MPs during eating, drinking, or respiration due to confusion. Fishes uptake plastic particles accidently or passively and via food chain by trophic level transfer (Roch et al. 2020). Fish can suck and swallow the MPs into their body. Fishes swallow them considering them as food due to their color and size. They swam toward them and were captured to swallow (Ory et al. 2018). If MPs are swallowed fishes chew and engulf them. Chewing time may increase if MP pellets are covered with food. Fishes may spit them out after identifying them by their oropharyngeal system. Some fish show sucking behavior. They suck the water through the mouth and transfer it to the gills for oxygen uptake, which increases the chance of MPs uptake. During this suction, some microfibrils may also enter into the gut. These microfibrils are stuck at the gills leading to the blockage of the gills. Often, some of these microfibrils are collected in the mouth of fishes and spit out by a process called coughing. While coughing, pressure is created in the mouth against water flow and microfibrils and mucus are spit out. Some MPs and microfibrils also remain in the gills and stomach after spitting and are then transported to body's other parts (Li et al. 2021).

Mainly MPs enter the body via swallowing (Wu et al. 2020). On the other hand, a study showed that MPs are present both in the body and head which indicates that both gut and gill are its routes. MPs which are present in the gill can be removed via filtration so they cannot penetrate the gill filaments but, in the gut, MPs can stay for a long time so the main route of MPs entry is the gastrointestinal tract (Su et al. 2019). Plastic ingested by fish has been

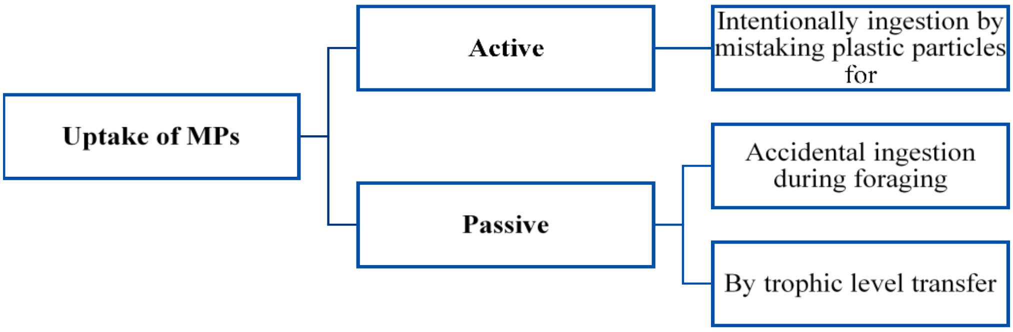

Fig. 3: Uptake routes of MPs in fish (Badea et al. 2023; Sulaiman et al. 2023).

confirmed in several species, and pollutants of ingested particles can move into the food chain and food web (Philips and Bonner 2015). Due to the small size of MPs, these are ingested easily by fish and ingested in more quantities. This small size makes their availability for the domain of animals, from the range of plankton feeders to the large filter-feeding fishes (Blesseling et al. 2015). The size of microplastic particles is considered the key factor for determining the biological availability of MPs for fish (Wright et al. 2018). More recently it has been reported that an abundance of plastic occurs in the intestine rather than the stomach in some fish species (Silva-Cavalcanti et al. 2017). The relationship between the habitat of freshwater fish, fish-feeding traits, and plastic pollution was studied, it was observed that fish collected in densely urbanized areas ingest MPs more frequently concerning those in less urbanized areas (Sanchez et al. 2014). Microplastic ingestion was not detected in those fish that were collected from low-impacted sites, and the hypothesis behind it is that urbanized areas have more wastewater treatment plants (WWTP) that are the basic source of MPs. Fishes with large gap sizes can ingest larger or maximum MPs. Feeding behavior also relates to ingestion. Microplastic exposure concentration and fish consumption rate have a close relationship. It also has been studied that microplastic ingestion can be increased if related to feeding cues (Kim et al. 2019).

Due to an increase in body size, trophic position (TP), and gape size, MPs prospectively accumulated in the gastrointestinal tract (GIT). Some fishes like *Micropterus salmoides* had more microplastic levels in GIT as compared to fishes of lower TP. Many factors are also involved in the prediction of microplastic ingestion. Stomach fullness could be a relevant factor in predicting the ingestion of microplastic. Notable factors were certified that can predict the ingestion of plastic. The basic factors that were analyzed are fish length (Delta), trophic level, region and trophic guilt. The difference in the ingestion of MPs can relate to sex, this could be because males and females have different body sizes (sexual dimorphism), which might be the real underlying cause of differences in MPs distribution (Horton et al. 2018; Su et al. 2019). After ingestion of MPs, these can be transported through absorption or adsorption into the gut, and supplements or additives can be transported from MPs into the tissues of the fish (Rochman et al. 2013). After ingestion of plastics, these MPs cross the barrier of the intestine and then transfer into and store up in the tissues. Most research on plastic ingestion focuses on how MPs build up in the digestive system GIT. However, the digestive system is connected to the outside environment through the mouth and anus. So, it is considered "external" even though it's inside the body. Some studies have found MPs in other parts of the body of freshwater fish as in the muscles, liver and brain (Abbasi et al. 2018). The aquatic plants can absorb and take in tiny plastic pieces, which can be later eaten by fish that feed on those plants, increasing the chances of fish plastic ingestion (Kalčíková 2020).

3. EFFECTS OF MICROPLASTICS ON FRESHWATER FISHES

MPs affect the physiology of various organs such as the liver, kidney and gut of freshwater fish. They also cause biological effects on genes, the immune system, the reproductive system, and behavior (Fig. 4). Comprehensive research on the impact of MPs on various freshwater fish species is presented in Table 1.

3.1. Pathophysiological Effects

3.1.1. Gills: Adhering microplastic with gill tissue cause irritation, which in response increases mucous secretion (Zheng and Wang 2023; Zeng et al. 2023). Under prolonged exposure, the ventilatory rate increases and then decreases (Xue et al. 2021). The alteration in breathing and abnormal mucous secretion can damage gas exchange, and by the osmo-respiratory compromise, diffusive ions may be lost (Wood and Eom 2021). Gills tissue damage due to MPs includes slough, fusion, hyperplasia, desquamation and clawing tips on the secondary lamellae, as well as epithelium lifting and cell proliferation (Limonta et al. 2019; Wang et al. 2022).

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Table 1: Studies on the effects of MPs on different Freshwater fish species

Fish species	Study Location	Effects	MP type	Conc. Of MPs	References
Labeo rohita	Gangasagar Pond, Darbhanga, India	All doses caused gills and mouth swelling, Gut mostly affected	LDPE	2, 20 and 200mg/L	Jandu and Vashishat (2021)
	River Ravi, Lahore, Pakistan	Intestinal swelling, lipid deposition, necrosis of muscle fibers, loss of filtration capacity of liver	PVS, PP, PS, PE	-	Raza et al. (2023)
Catla catla	Government College University, Faisalabad, Pakistan	Increased fat and low protein content, inhibited growth, lower nutritional quality and abnormalities in gut	-	0.5%, 1%, 1.5%, 2%, 2.5%	Rashid et al. (2023)
	Dhaka, Bangladesh	Adversely impact intestinal cells	PS, PP, N, HDPE and Ny	-	Nath et al. (2024)
Cyprinus carpio	Al Azhar university, Egypt	Necrosis, degeneration, formation of vacuoles in the retina and tectum	PE	-	Hamed et al. (2022)
	Henan, China	Inhibited growth, alteration in antioxidant enzyme activities formation of vacuoles in the liver cells.	PVC	10%, 20%, 30%	Xia et al. (2020)
	Ordu University, Türkiye	Oxidative damage to gills, affected malondialdehyde levels also glutathione levels increased	PP	1.0g/L and 2.5g/L	Yedier et al. (2023)
	People's Republic of China	Abnormalities in hepatopancreas function and excessive reactive oxygen species caused inflammation	PP	1000ng/L	Cui et al. (2023)
	Iran	Altered biochemical and immunological factors	MPs	-	Banaee et al. (2019)
Ctenopharyngodon idella	Shanghai Ocean University, China	Oxidative stress, metabolic disorders, liver cell vacuolization, decreased weight	MPs	1000 and 100µg/L	Liu et al. (2022)
	China	Harm intestine, gut microbe's alterations, liver inflammation, growth inhibition	PS	100µg/L, 500µg/L	Hao et al. (2023)
	Wujiang, China	Highest damage to the gut causing harm to tissues, formation of vacuoles and cell death of the enterocytes)	MPs	100 and 1000µg/L	Jia et al. (2024)
Oreochromis niloticus	Egypt	Increase in reactive oxygen species, altered antioxidants, Oxidative stress, Formation of DNA fragments	MPs	1mg/L, 10mg/L and 100mg/L	Hamed et al. (2020)
	Indonesia	MPs accumulate in the gut, gills, liver, gonads, muscles, blood	PE	-	Aryani et al. (2021)
	Gårdsfisk AB, Sweden	Liver cell vacuolization, and breakdown, increased growth of gill cells, Cellular degradation of intestine, degradation of muscle fibers and pancreatic tissue	FFBP	-	Kardgar et al. (2024)
	Al Azhar University, Assiut branch, Egypt	Hemato-biochemical parameters alterations, accumulation in tissues, anemia, mortality of juveniles	-	1, 10, 100mg/L	Hamed et al. (2019)
		Toxic impacts on microbiota community, metabolism, gene expressions and thus fish growth	PE	-	Lu et al. (2022)
	Atoyac River Basin, Mexico	High MPs accumulation	PA, PES, and cellulose		Martinez-Tavera et al. (2021)
Channa maculata	China	Alters gills structure affects liver and causes oxidative stress, Alters the activity of antioxidant enzymes and suppress inflammation causing gene expression	PP	-	Wang et al. (2022)
Oreochromis mosambicus	Tamil Nadu, India	Disrupts acetylcholinesterase activity fluctuates homeostasis, Damage DNA as a result induces Genotoxicity	-	100, 500, and 1000mg/kg	Jeyavani et al. (2023)
	-	oxidative stress	PLA	100µg/L	Bao et al. (2023)
Danio rerio	Iran	Gut alterations such as villi lesions, epithelial detachment, etc., Necrosis of liver infiltration of liver and accumulation of lipid droplets in the liver	PU		Dinani et al. (2021)

Carassius carassius	Republic of Korea	Acetylcholinesterase activity decreased in the gills, liver, and intestine, Immunoglobulin and lysozyme immune response decreased	-	-	Choi et al. (2023)
		affects the hematological physiology and antioxidant	PA	0, 4, 8, 16, 32 and 64mg/L	Choi and Kim (2023)
Cirrhinus mrigala	South China Agricultural University.	Affects growth and survival, and accumulations in the intestine, Alters the antioxidant enzyme activity, and detoxification gene's expression.	-	-	Wang et al. (2023)
Atropus atropos	Persian Gulf, Iran	Hazardous effect	PE and Ny	0.147/g	Esmaeilbeigi et al. (2023)

MP=Microplatics, PVS=Polyvinyl chloride, PP=Propylene, PS=Polystyrene, PE=Polyethylene N=Nitrile, HDPE=High-density polyethylene, Ny=Nylon, LDPE=Low density polyethylene, FFBP=Fossil fuel-based polymers PA=Polyamide, PES=Polyester, PU=Polyurethane, PLA=Polylactic aci.

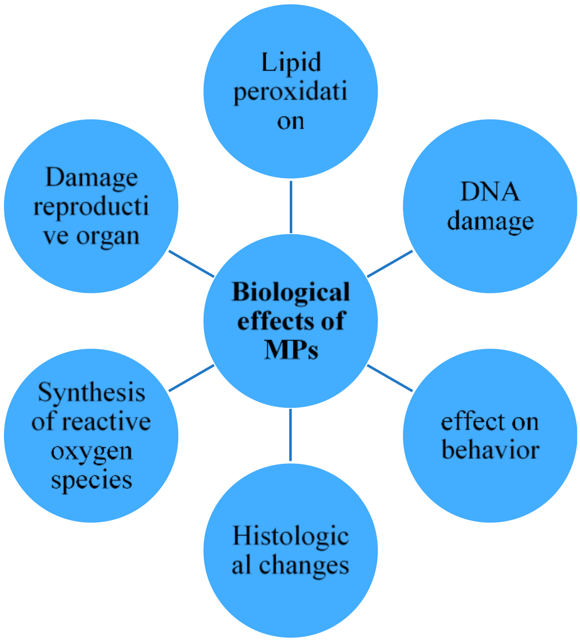

Fig. 4: Biological effects of MPs in fish (Bhat et al. 2024).

3.1.2. Gut: Microplastic ingestion can harm freshwater fish's gastrointestinal system, including obstruction, inflammation, and tissue damage, eventually leading to decreased metabolism and nutrient uptake (Verma and Prakash, 2022). The accumulation of MPs in fish causes several harmful effects, such as damage to the mucosa, increase in permeability, inflammation and disruption in metabolism, and microbiota dysbiosis (Jin et al. 2018; Xie et al. 2021; Bao et al. 2023). It is found that ~70µm MP exposure to zebra fish causes damage to intestine, including cracking of villi and splitting of enterocytes (Lei et al. 2018). Microplastics decrease juvenile digestive enzyme activity and stimulate the gut immune response (Hirt and Body 2020). MPS could exist in the guppy gut and induce the enlargement of global cells. The young guppy acquired dysbiosis, with an elevated abundance of Proteobacteria and a lower abundance of Actinobacteria, after exposure for 28 days to PS microspheres (32–40 µm, 100 and 1000µg/L). Exposure of larval zebrafish to PS MPs (5 and 50µm, 1000µg/L) for seven days reduced microbiota richness in the gut and significant variations in the abundance among the various geniuses (Wan et al. 2019). Zebrafish research examined 21 days of exposure effects to PS nanoparticles at low doses (5, 50, and 500µg/L) showed a reduction in Proteobacteria abundance, an increase in Fusobacteria abundance, and a decrease in Bacterium diversity (Qiao et al. 2019). Microplastic exposure to *Catla catla* for 90 days resulted in the alteration in gut structure, such as disruption of the mucosal layer and fused villi (Rashid et al. 2024).

3.1.2. Liver: Histopathological sections from *Oeochromis. mossambicus* exposed to MPs show signs of oxidative stress in liver cells due to the following manifestations: increased generation of ROS levels during exposure, changes in the antioxidant biomarker levels, increased lipid peroxidation, DNA fragmentation, cell death, necrosis, vacuolation and atrophy of dilated sinusoids in liver tissues (Jeyavani et al. 2023). MPs Exposure of 5µm from 0-7 days causes inflammation and lipid accumulation in zebra (Lu et al. 2016). Accumulation of MPs can disrupt the metabolism of lipids and energy and can also stimulate oxidative stress in the liver of zebrafish (Zhao et al. 2023). Various studies have shown that the accumulation of MPs can damage the liver of freshwater fishes such as tilapia, grass carp due to an increased number of reactive oxygen species leading to oxidative stress, peroxidation of lipid and lowered antioxidant enzymes (Subaramaniyam et al. 2023). In another study, a zebra fish was exposed to 50nm to 4.5µm for 21days leading to formation of vacuoles in the liver indicating liver damage (Martínez-Álvarez et al. 2024).

3.1.3. Kidneys: Toxicological studies conducted on freshwater fish reported that kidney microplastic exposure leads to physical harm to vital organs, including kidneys (Banaee et al. 2024). In freshwater fish, the main symptoms associated with microplastic uptake were changes in kidney function, including decreased filtration capacity and disturbances in ion homeostasis (Zink and Wood 2024; Hasan et al. 2024). These MPs have been found to induce reactive oxygen species in the kidney parenchyma of freshwater fish and compromise renal functions (Espinosa et al. 2018; Sayed et al. 2020).). Some studies have shown that MPs affect the renal system of freshwater fish, leading to inflammation, damage, and dysfunction. Analysis of MPs' general effects on freshwater fish's renal biomarker has shown that it leads to stressed and damaged kidneys. There are several histopathology changes, especially in the kidney tissue of freshwater fish species exposed to MPs, including tissue lesions and cell morphologies (Meng et al. 2022). In another study, when a zebrafish was subjected to MPs, it led to kidneys accumulation and triggered oxidative damage, consequently weakening immune systems (Yang et al. 2024).

3.2. Biological Effects

3.2.1. Immune system: Freshwater fish's immune systems have been illustrated to be heavily influenced by MPs. Scientific studies have shown that MPs may alter the way immune genes are expressed in fish, which impacts the manner fish can react to immunity. For example, MPs were found to enhance the expression of some immune-related genes, such as factor-alpha of tumor necrosis (TNF-α) and hypoxia-inducible factor-1α (HIF-1α), while inhibiting the expression of other genes, such as interferon (IFN), in a study done with yellow catfish (*Pelteobagrus fulvidraco*) (Li et al. 2021). A different study discovered that MPs can cause reactive oxygen species to be produced. These reactive oxygen species can then cause the creation of danger-associated molecular patterns (DAMPs) and damage toll-like receptors (TLRs), which can cause immune cells to become inflamed (Yang et al. 2022). This has been observed that the effects of MPs of different sizes on zebrafish immune responses vary. The study has suggested that tiny MPs, consisting of 5μm polyethylene microbeads, might modify immune gene expression and lead to modifications in the immune function response, such as elevated expression of genes linked to immune system responses and oxidative stress.

Larger MPs, including 100μm polyethylene microbeads, are known to cause severe immune system responses, such as increased reactive oxygen species production and interference on toll-like receptors (Parker et al. 2021). After the uptake of microplastic, at transcriptional level the immune cells make effective modulation. For instance, the levels of IL-1α, IL-1β and interferons in the Zebrafish gut were significantly up-regulated after exposure to PS, MPs. (Jin et al. 2018). There are many alterations to zebra fish mucous of the intestine such as detachment of epithelial cells, hypersecretion of mucus, and higher activation of neutrophil). Response of the immune system, epithelium intactness, and lipid anabolism and catabolism related genes are moderated (Limonta et al. 2019).

***3.2.2. Reproductive effect*:** Reproductive toxicity includes harmful effects on several stages of the reproductive cycle in animals, such as the formation of gametes, gamete and egg quality, rate of fertilization, production of the egg, and motility rate of sperm (Sussarellu et al. 2016; Sharifinia et al. 2020). Researchers have investigated the harmful effects of MPs at different stages of the fish reproductive cycle. When female fishes were exposed to MPs, the resulting effects were the reduced rate of pregnancy and counts of the embryo (Malafaia et al. 2022). When Zebrafish and medaka fish were given to MNPs showed a decrease in production of mature sperm and eggs which caused reduced fertilization rates (Wang et al. 2021; Cormier et al. 2021). Exposure to MNPs could triggerfish gonads to form excessive reactive oxygen species (ROS). Increased ROS is an indicator of reproductive harm caused by MNP exposure (Hu and Pali 2020). This ROS leads to cell death in reproductive organs (Qiang and Cheng 2021).

3.2.1. Genotoxicity: Microplastics, especially polystyrene containing size 1Micro meter can show genotoxic effects in freshwater fishes by damaging the DNA. The accumulation of MPs in fish bodies leads to transportation to DNA via the cell membrane, causing damage by increasing DNA tail length, tail moment, and tail density (Berber 2019; Kushwaha et al. 2024; Menezes et al. 2024). MPs exposure triggers the excessive production of reactive oxygen species (ROS) leading to oxidative stress that interferes with biomolecules such as proteins, lipids and DNA, causing genotoxicity (Jeyavani et al. 2023). Exposure to MPs further initiates inflammation, leading to an increased number of macrophages, activation of NF-κB and cytokines IL-6. NF-κB activation enhances nitric oxide production which reacts with superoxide to form peroxynitrites that cause significant DNA damage. Moreover, MPs impair the DNA repair mechanism (Fig. 5) by decreasing heat shock proteins (Tagorti and Kaya 2022).

3.2.2. Behavioral effects: The exposure of microplastic fibers to goldfish (*Carassius auratus*) at 1000 items/L after 2 hours caused an increase in the coughing behavior of fish, while the predatory behavior was decreased by 53% after 45 days of exposure, and the feed intake of fish was decreased significantly (Liang et al. 2023). Zebrafish (*Danio rerio*) larvae were sub chronically exposed to MPs (2mg/L). Results demonstrated that MPs affected the different behavior patterns in the larvae; and decreased their speed, swimming behavior, and avoidance behavior. Overall, it reduced the survival of the larvae (Santos et al. 2020). After exposure to polystyrene and polyethylene MPs in adult zebrafish for 20 days, the daily activity of the fish altered; especially its movement activity. Other than that, it was also observed that the normal diurnal pattern of the fish also got disrupted, leading to increased activity during the night (Limonta et al. 2019). Zebrafish were exposed to polystyrene MPs at the embryonic stage, later on,

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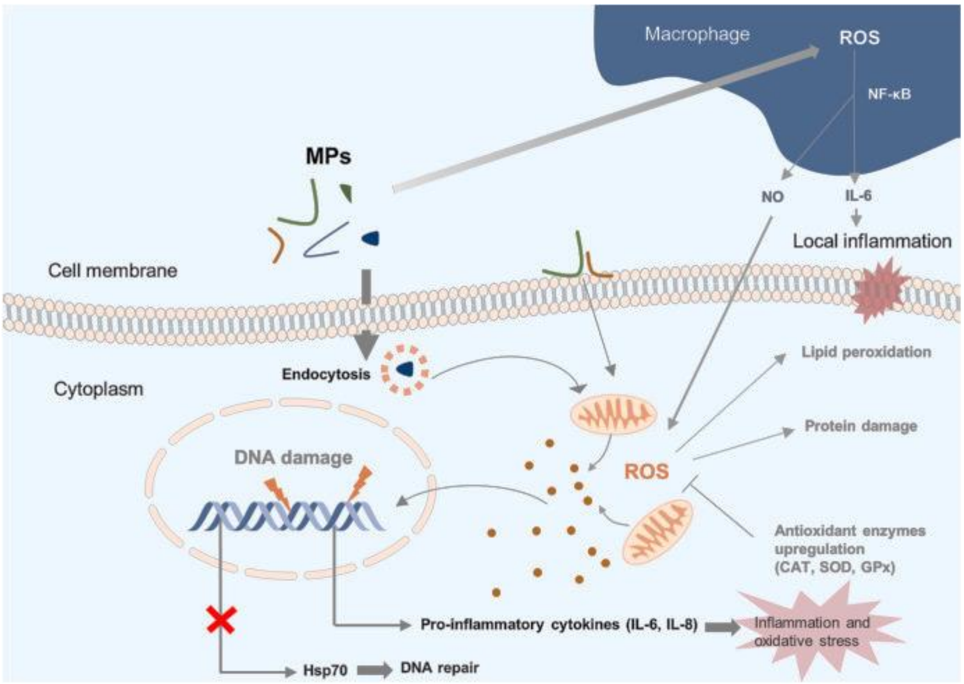

Fig. 5: The proposed mechanism of MPs-induced genotoxicity (Tagorti and Kaya 2022).

at the adult stage, the neurodevelopmental responses were examined; results showed that fish showed hyperactivity at the later stage of life. Also, oxidative stress and nervous system gene expressions increased, other than that, DNA hypomethylation occurred, which confirmed the neurodevelopmental toxic potential of MPs; so overall, it changed the behavior of the fish (Im et al. 2022).

In an experiment, when zebrafish embryos after four hours of fertilization were subjected to MPs; MPs penetrated the stomach and intestine of the larvae. When a 1000µg/L microplastic concentration was given; it showed an alteration in the swimming behavior of fish (both the swimming distance and speed of larvae declined) (Qiang and Cheng 2019). When European perch (*Perca fluviatilis*) larvae were subjected to of MPs dose that was relevant to the environment at ninety micrometers, it altered various behaviors of the fish; feeding preferences (newly hatched larvae in the experimental group started preferring microplastic particles over natural food), decreased anti-predatory response, and the mortality rate due to predators increased. Locomotor activity also decreased (swimming rate and speed) (Lönnstedt and Eklöv 2016). Acute and chronic tests were conducted to study the impacts of polyvinyl chloride MPs on the fish *Etroplus suratensis*, in India. Results showed several behavioral alterations in the fish, including vibrating fins, unstable or shaking movement, and short and intense swimming (Vijayaraghavan et al. 2022). Microplastics adversely affect the predatory behavior of the fish, so the fish cannot differentiate between MPs and prey, ultimately accumulating in the fish organs (De Sá et al. 2015). MPs also reduce the swimming in fish due to which they cannot resist the water flow (Barboza et al. 2018). MPs altered the behavior of the fish due to decreased energy from feeding the indigestible particles, also, the movement of the fish was altered. Other than that, the escape responses of the fish were altered due to the ingestion of high microplastic concentrations (Nanninga et al. 2021). For seven days when zebrafish were subjected to MPs, results showed that swimming speed and distance increased 1.3–2.4 folds compared to the control group, and the active period of the fish also increased. Hence, the fish became hyperactive because of particle matter activation and higher estrogen levels (Chen et al. 2020).

4. BIOACCUMULATION AND BIOMAGNIFICATION OF MICROPLASTICS

Microplastic accumulation is directly related to fish's length that is why a higher number of MPs in females is associated with their extended length (Horton et al. 2018). Also, the accumulation of MPs in fishes is correlated to the difference in the morphology of the gastrointestinal tract. The shorter the GIT, the more excretion of MPs and less accumulation of MPs in the body of fish (Zheng et al. 2019). A study confirmed that a more complex stomach and intestine exhibit the chance of more accumulation of MPs as compared to others, especially those species that have coiled structured intestine (Jabeen et al. 2017; Huang et al. 2019). A study was done on Japanese medaka to test the bioaccumulation of polystyrene MPs, fish was subjected to two-micrometer fluorescent polystyrene microplatsics for 21 days. *Java medaka* showed more MPs bioaccumulation as compared to Japanese, and results also showed that MPs could accumulate in the intestine (Assas et al. 2020). MPs can accumulate in the different tissues of the red tilapia, and it was confirmed by this study, in which red tilapia were exposed to polystyrene MPs for 14 days and bioaccumulation followed this order of gut> gills> brain> liver. Trophic transfer of polyethylene

MPs on *Physalaemus cuvieri* tadpoles, tambatinga fish and mice was investigated. The tadpoles were exposed to MPs for 7 days, afterwards, the tadpoles were fed to fish for the same period, and at last, and these fish were given to mice. Results showed that accumulation of MPs occurred in all trophic levels and also induced behavioral changes in the mice (da Costa Araujo and Malafaia 2021).

Microplastics in the various tissues of the fish depend upon several factors including, particle size, exposure route, exposure concentration and time, fish species, and absorption pathway (Ding et al. 2018). A lot of studies concluded that microplastic bioaccumulation largely depends on particle size (Lu et al. 2016), with smaller particles that are easier to accumulate (Zitouni et al. 2021). 5µm easily enter the circulatory system via intestinal cells, while 5–150µm particles are modified in the intestinal membrane and then enter the circulatory system (Jovanovi´c et al. 2018). Gut is the most common part where MPs accumulate and then carried to other parts (Jovanovi´c 2017). Polystyrene (0.1µm) MPs showed significant bioaccumulation in *Oreochromis niloticus* (Ding et al. 2018). After accumulating in the brain MPs can directly impact the central nervous system while kidneys and livers get exposed to higher MP accumulation because of their role in detoxification and excretion of substances from blood (Kim and Kang 2015). A study on *Danio rerio* revealed that MPs first accumulate in the head and yolk sac (leading to metabolic disorders) of the fish and then later on, other tissues like pericardium (high accumulation related to cardiac toxicity), liver, pancreas, GIT (Pitt et al. 2018). 5µm polystyrene MPs bioaccumulation in *D. rerio* doubled as compared to 20 µm polystyrene MPs (Lu et al. 2016). Freshwater or marine water also influences microplastic bioaccumulation; freshwater fishes eliminate more water via urine as compared to marine so it results in higher bioaccumulation in marine water fishes when subjected to an equivalent dose of MPs (Lee et al. 2019; Assas et al. 2020).

Microplastics bioaccumulation occurs when the concentration of elimination of MPs is lower than uptake (Newman and Unger 2003; Wang et al. 2016). A study on plastic consumption was done on 427 species, results were in the following order: Marine water fishes > freshwater fishes > marine-brackish > brackish-freshwater. Among them according to feeding habits fishes showed the following order: carnivorous> omnivorous >herbivorous > algivorous > detritivorous (Azevedo-Santos et al. 2019). Another study conducted on 198 fish species showed contrasting results omnivorous > carnivorous (Sequeira et al. 2020). Microplastics accumulation in fish species sampled from urban areas showed more as compared to rural areas because urban areas are near wastewater or MPs sources (Bellas et al. 2016; Jabeen et al. 2017; Chan et al. 2019; Arias et al. 2019). A study was conducted in Pakistan to investigate the MPs the MPs transfer across the food chain in the two freshwater bodies: River Ravi and a rearing pond that was supplied with groundwater. Samples were taken from air, water, sediments, plankton, fishes and birds, Results concluded that bioaccumulation was evident at every trophic level (Qaiser et al. 2023). Mode of ingestion also affects accumulation of MPs in fishes and other organisms; depends upon feeding strategy like engulf, swallow or filtration (Li et al. 2020), the morphology of intestine and habitat degradation by plastic (Jabeen et al. 2017; Feng et al. 2019; Yuan et al. 2019). Additional factors for variations in bioaccumulation include fish size, environment, biological traits and place of sampling (Ferreira et al. 2018; Horton et al. 2018; Yuan et al. 2019). It is also hypothesized that fishes ingest those MPs readily that resemble their prey (Zheng et al. 2019).

5. MICROPLASTICS AS CARRIERS OF CONTAMINANTS

Studies have confirmed their ability to facilitate the transportation of other compounds because they can adsorb drugs (e.g., acyclovir, atenolol, sulfamethoxazole, and ibuprofen), heavy metals (As, Cd), pesticides (buprofezin, imidacloprid), antibiotic-resistant genes and microorganisms. MPs are suitable spots for antibiotic-resistant genes and bacteria (ARGs, ARBs) (Imran et al. 2019; Lu et al. 2019). The aging properties of MPs and how they behave as carriers for antibiotic-resistance genes were studied. The ability of MPs to accumulate and transport ARGs is enhanced as MPs undergo various physical and chemical changes with time (Su et al. 2021). Biofilm production plays a complex role in this process on the surface of aged MPs. Biofilms provide a conducive environment for vertical gene transfer (between generations of bacteria) and horizontal gene transfer (between different bacterial species). As a result, MPs act as hotspots for the production and diffusion of ARGs in various environments. MPs have great absorption potential, which means that they can carry and accumulate organic chemical contaminants (OCCs) and metals on their surfaces from the lakes and rivers in their vicinity. (Lee et al. 2014; Chen et al. 2019). Prior research has found that MPs can absorb different kinds of hydrophobic organic pollutants (HOP). Hydrophobic organic pollutants exhibit polarities in the range of 3.3–9 and are most repeatedly reported as associated with MP surfaces. According to (Llorca et al. 2020) these pollutants include PCBs, perfluoroalkyl substances (PAFSs), (polycyclic aromatic hydrocarbons (PAHs), hexachlorocyclohexane (HCHs), pesticides (e.g. DDT, OCP), and bisphenol analogs (BPAs). A study was conducted to assess the combined effects of MPs and Cu, Zebrafish larvae were exposed to MPs combined with Cu for 14 days after fertilization. As a result, the survival rate of larvae was reduced because of disturbed locomotion and reduced anti-predatory response (Santos et al. 2021). In another study where MPs and paraquat (herbicide) were exposed to common carp for 21 days, results showed an increase in albumin levels and changes in blood biochemical parameters. It also shows that if there is an increase of MPs in water, parquet toxic effects will also increase (Nematdoost et al. 2017). The combined effect of a pharmaceutical (diazepam) and MPs was investigated on the medaka for 7 days, results showed that combined MPs and diazepam suppressed the social behavior of medaka. So it was determined that MPs can increase the adverse effects of contaminants (Takai et al. 2022).

6. CONCLUSION

Microplastics from different sources such as industries, fabrics, washing areas, plastic waste from households, etc. enter the aquatic environment and adversely affect aquatic life. Effects are shown at all levels from molecular, cellular, and organismic levels. Even trophic level transfer also occurs, which can be more adverse for humans. Thus, new methods for proper disposal, management and identification of MPs need to be discovered.

Research Gaps and Future Recommendations

This review highlights the effects of MPs on freshwater fishes in detail, so proper waste management systems should be developed to save freshwater from these hazardous particles. Different new and traditional methods should be employed to eliminate these particles from the environment, such as heat and salt treatment, smashing, recycling of plastic and dumping, electrolytic methods, etc. As the size of MPs is very minute, it is challenging to quantify them; therefore, for the exact measurement of the MPs in lakes and rivers, new procedures must be devised. There is a need to do further studies on freshwater microplastic sources, effects, and quantification in environmentally relevant concentrations. Other than that, a detailed trophic-level study should be done on humans to discuss the impacts of MPs on them.

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