

# Detrimental effects of microplastics in aquatic fauna on marine and freshwater environments – A comprehensive review

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## ABSTRACT

The world is ever evolving and new technologies are popping up everywhere. New inventions and discoveries have created a better world, but not a sustainable one. The whole earth is drowning in various pollutants and garbage. Plastic pollution has garnered sufficient attention and there are various teams and organizations working toward cleaning our beaches, parks, and environment. However, all these actions will not suffice as plastics have trickled down into microplastics, which are posing a greater threat to our water systems and aquatic fauna. Several ongoing researches focus on marine microplastics, while only 13% of studies are on freshwater. Research on microplastics is now on the rise, with new strategies and restrictions being put into place to curb its accumulation in our marine and freshwater environments. In a recent study, microplastics were found to be present in human blood with. Out of 22 people tested, 17 test subjects had microplastics present in their blood. This review focuses on the adverse effects of microplastics in marine and freshwater ecosystems, with special focus on aquatic fauna.

## 1. INTRODUCTION

Aquaculture, commonly known as aqua-farming, is regarded as the “agriculture of the oceans.” It is the cultivation (growing, rearing, breeding, and maintenance) and harvesting of algae, aquatic plants, fish, crustaceans, mollusks, and other organisms in varied aquatic environments that include ponds, lakes, rivers, and estuaries [1,2]. Aquaculture has significant socioeconomic value [3-5]. The FAO reports that in 2018, the total export value of fish traded internationally was US \$ 164 billion [6]. India ranks second in aquaculture and third in fisheries in the world. The fishing industry employs millions of people and is a significant contributor to the country’s food security [7-9]. Fish and fish products contribute significantly to our diet as they are crucial sources of high-quality proteins and essential amino acids. They also consist of polyunsaturated fatty acids and micronutrients, such as vitamins and minerals [10].

Freshwater aquaculture involves the breeding and raising of aquatic organisms which include fishes, prawns, shellfish, and crabs and aquatic plants. They are reared in rivers, reservoirs, ponds, lakes, and other inland waterways – which include brackish water for economic purposes. Freshwater aquaculture plays a pivotal role in the

aquaculture industry [11]. India’s fish production comprises nearly 66% (two-thirds) of the country’s total fish production from both culture and capture sources, with an estimated total fish production of 6.24 million metric tons in 2018. In the fish farming sector, freshwater aquaculture plays a key role and is a major contributor, as marine finfish culture is rarely practiced on a large scale. About 12.8% of the total animal protein consumed in India, comes from freshwater fishes [12] [Figure 1].

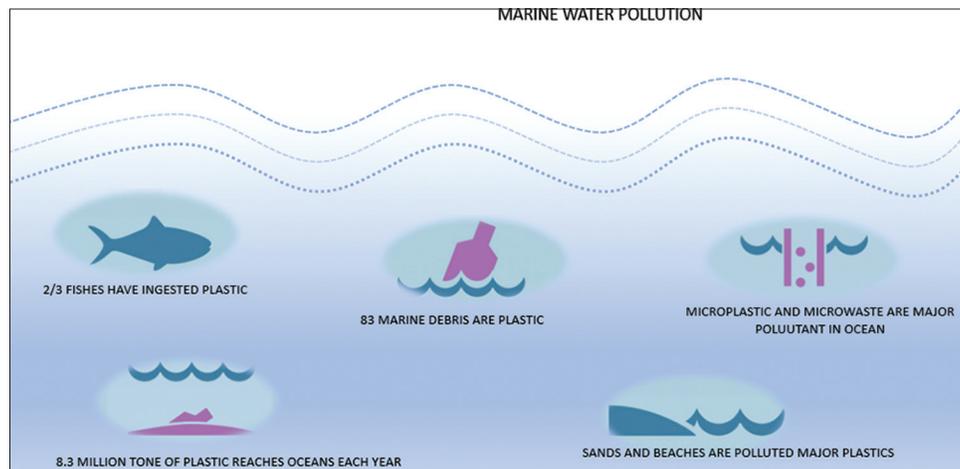
Our planet is limping toward an impending environmental catastrophe caused by our addiction to single-use or disposable plastic. If this trend continues, by 2050, 20% of the world’s total oil consumption will be accounted for by the plastic industry [13]. The great Pacific patch which was discovered in 1997, by Captain Charles Moore, was the beginning of what Captain Moore quoted as “The Ocean is downhill from everywhere – the principal repository for vagrant plastic waste [14].” Of all the plastic waste generated, the majority (79%) is dumped into landfills, while 12% is incinerated and a measly 9% is recycled. A recent global study found that the most common plastic waste consists of cigarette butts (filters contain tiny plastic fibers), beverage containers, bottle caps, grocery bags, food wrappers, straws, stirrers, etc. If these alarming trends persist, our oceans are estimated to contain more plastic waste and microplastic particles than fish and other aquatic organisms by 2050. Developing efficient and cost-effective initiatives to counter the widespread damage caused by plastic waste to the environment are the need of the hour [15,16].

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**Figure 1:** Major pollutants of marine and ocean water.



**Figure 2:** Primary source of microplastics.

Although plastic debris has many shapes and sizes, those <5 mm long (the same approximate size as a sesame seed) are regarded as “Microplastics.” Microplastics are the most prevalent plastic debris found in our marine environment (oceans, rivers, and lakes, including the Great Lakes). Certain microplastic particles are derived from primary sources, that is, they are deliberately produced as microplastics. Other varieties emanate from secondary sources; they are the byproduct of weathering and fragmentation of bigger plastic objects [17-19]. As of 2018 [20], plastics constitute 12.20 % of municipal solid waste of the world. India is one among the few countries that have policies to minimize microplastic waste, although currently the major bans focus on larger plastics [21].

Microplastic research has been more focused on the oceans and seas. Studies relating to freshwater microplastics are <4% [22]. The distribution of microplastics is highly heterogeneous, and its abundance in freshwater is comparable to that of the marine environment as well [23,24]. Wastewater treatment plants (WWTPs) are one of the prominent and primary underlying sources of microplastics, while terrestrial sources contribute to the rest [25-30]. WWTPs are capable enough to remove up to 95% of microplastics [31] and tertiary treatment has a removal efficiency of up to 90% of finer particles of size larger than 10 mm [32]. In spite of this, a considerable amount of microplastics are released into the natural water system through WWTPs.

Primary and secondary microplastics are widespread in sediment, water, and biota of both marine and coastal environments. Microplastic fragments are analogous to the size of feed elements and closely resemble the appearance of phytoplankton, zooplankton, and suspended particles [33]. This leads to the ingestion of synthetic

microparticles by invertebrates. The suspension feeders and benthic organisms feed on the indistinguishable microplastics from the contaminated water and bottom sediments, mistaking them to be food particles. Organisms seen to ingest microplastic fibers and fragments include barnacles, lugworms, amphipods, and tiny organisms such as filter-feeding zooplankton which are placed at the bottom of the food chain [34-36]. More than 1401 marine species come in contact with marine plastic debris in numerous ways [37] [Figure 2].

Besides the physical risk from plastic litter in the marine environment, there is also the risk of marine organisms ingesting the chemicals that are not only in the plastics but also on the surface water. The plastic particles in the ocean have been found to have the ability to attract organic non-dissolving chemicals that contain known toxic substances. They also have the tendency to absorb Plaster of Paris and heavy metals [38,39]. This has paved the way for increasing studies, exploring plastics' actions concerning marine organisms and toxic chemicals. This is a result of rapid urbanization and encroaching residential complexes. The biodiversity of a region is highly impacted by its water resources. The lakes that exist now are heavily polluted with plastic debris and also with industrial and urban run-offs. This has an adverse effect on the aquatic fauna as they are subjected to toxic exposure and end up consuming microplastics due to fragmentation [Figure 3]. This review aims to shed light on the impact of plastic and microplastic pollution in the entire marine and freshwater environment, with particular attention to aquatic fauna. The central question is to ultimately understand how this plastic debris would travel up along the food chain and eventually end up on our plates. The World Environmental Day theme of 2021 was on ecosystem restoration, “Reimagine. Recreate. Restore.” which is also much relevant to the present review. Microplastic pollution and studies on how to curb it is the need of the hour.

## 2. MICROPLASTICS IN FRESHWATER SOURCES

The United Nations has declared plastic pollution as one among the critically emerging environmental issues of our times [40]. The major share (nearly 80%) of plastic waste in the ocean is carried there by rivers [41,42]. Only 13% of studies focus on plastic pollution in the freshwater environment [43]. In a study conducted at 4 lakes of the Great Lakes system, plastic particles were documented down to the smallest size ever reported of 106–333  $\mu\text{m}$  [44]. A study conducted at Vembanad lake which is a Ramsar site in India, on microplastic pollution in the sediments, revealed a mean abundance of  $252.80 \pm 25.76 \text{ m}^{-2}$  (96–496

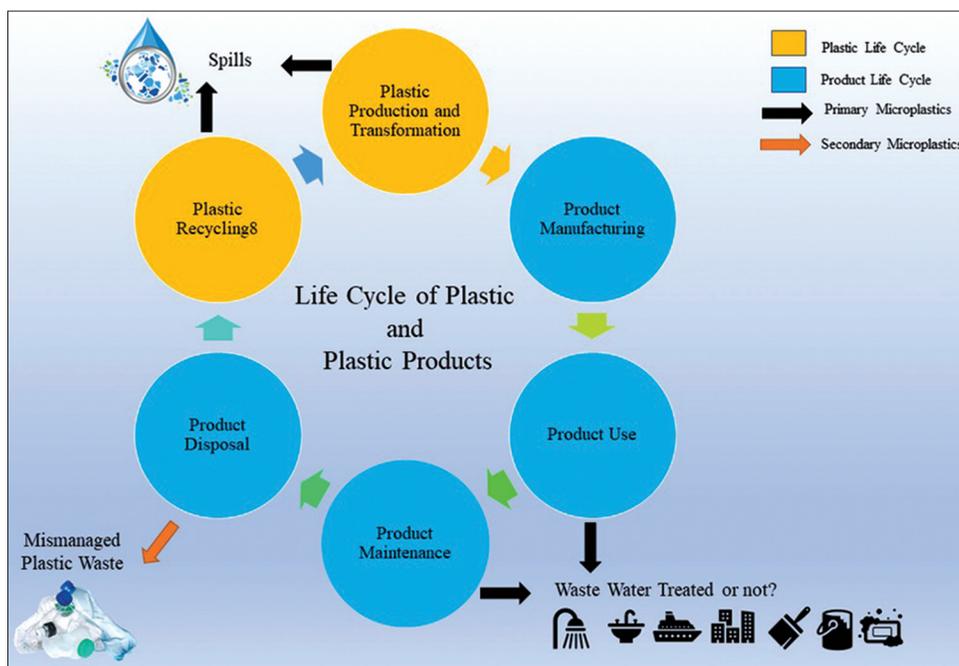


Figure 3: Lifecycle of plastic and plastic products.

particles  $m^{-2}$ ) of MPs from all 10 sample sites studied [45]. A research work by Julien *et al.*, to determine the microplastic stocks and fluxes in the Lake Geneva Basin, taking into account two very distinctive approaches: (a) A top-down approach which fixates on modeling plastic fluxes based on the socioeconomic activities and (b) a bottom-up approach which would utilize field measurements to estimate the plastic fluctuations. The release evaluations based on top-down modeling reported a mean value of 49 tons/year, and the bottom-up approach reported a mean value of 59 tons/year of MPs being released into Lake Geneva with an average of 55 tons of microplastics being released Lake Geneva every year [46]. A study conducted at the Mvudi River, in Limpopo province of South Africa focused on the river ecosystems, which are a key component of the global water cycle. The samples were collected from an intermittent stream in the Mvudi River during three seasons, at five different sites. The various study periods were cool-dry season (June 2019), hot-dry season (September 2019), and hot-wet season (February 2020). In this study, a total of 2406 microplastic particles were collected. The particles collected varied with the seasons, with 199 items collected during cool-dry season, 217 items during hot-dry season, and 1990 items collected during the hot-wet season. Fibers and microbeads (white color in abundance) majorly constituted the samples. The mean MPs density was highest during the hot-wet season and lowest during the cool-dry season. Due to the high prevalence of raw sewage in the entire river, the effect of wastewater and sewage was not evident [47].

### 3. MICROPLASTICS IN CRUSTACEANS AND ZOOPLANKTONS

Microplastics are constantly taken up all aquatic organisms and easily travel up the food chain. In a study led by Ping Yu, on *Eriocheir sinensis* (Chinese Mitten Crab) exposed to microplastics, the activities of glutathione, glutamic-oxaloacetic transaminase, glutathione peroxidase, and superoxide dismutase (SOD) increased in the specimens exposed to low concentrations of polystyrene microplastics (40 and 400  $\mu g/L$ ) when compared to control. In organisms exposed to

higher concentrations of 4000 and 40,000  $\mu g/L$  microplastics, the levels decreased accordingly [48]. Another research done on the freshwater shrimp *Neocaridina palmata* to study the retention time and egestion of microplastics revealed that the shrimp had ingested fluorescent polyethylene beads, polystyrene beads, and fluorescent polyvinyl chloride fragments in a concentration-dependent manner. Mostly ingestion of smaller beads 38–45  $\mu m$  was detected. The shrimp had egested 59% of beads and 18% of fragments within a span of 4 h [49].

Even though most zooplankton evacuates microparticles in a matter of hours, some have been discovered to have retained the undigested particles for as long as 7 days [34]. The nutritional intake and reproductive output of zooplankton were found to have decreased significantly due to their ingestion of polystyrene particles. The zooplankton can usually survive on up to 40% less real food [50,51]. Besides receiving no energy from non-nutritional microplastics, zooplankton and other organisms deal with food shortages. They are also unable to instinctively reduce their metabolic rate during periods of starvation, which directly results from a diet of microplastic beads [50].

The ingestion of synthetic microbeads by zooplankton limits its feeding, growth, and survival by causing heavy hindrance in the digestive tract [35,51,52]. A work by Castro focused on the effect of polyethylene microplastics of the size 40–48  $\mu m$  on *Daphnia magna* newborns. The mobility of organisms was not altered due to exposure, but *D. magna* promptly ingested polyethylene MPs in the first 24 h of exposure. The microplastics did not have any influence on the molting process, but the availability of feed did intervene with the number of molts produced [53]. A study focusing on the effects of food supply and temperature in the ingestion of microplastics by two model organisms *Daphnia magna* and *Daphnia pulex* was conducted by Hoffschroer. An increase in temperature increased the MPs uptake in the organisms. In response to elevated temperatures, low oxygen availability, and low food supply, the complex regulation pattern of *Daphnia*'s filtration current increased the frequency of leg movement, which led to increased ingestion of MPs [54].

#### 4. MICROPLASTIC STUDIES IN MOLLUSKS AND AMPHIBIANS

Pastorino and his team investigated microplastic pollution in Lake Iseo using the Zebra mussel as a bioindicator. It was found that the zebra mussels retained particles >149  $\mu\text{m}$ . The retention efficiency of MPs by mussels was 6% for 0.5  $\mu\text{m}$  microspheres and 29% for 1.0  $\mu\text{m}$  microspheres, respectively. It was higher, 87–100% for all larger microspheres. The most prominent color of MPs in samples was blue (60%), and the most prevalent chemical composition of MPs in samples was 45% of polyethylene terephthalate [55]. A study was conducted using *Anodonta trapesialis* – a freshwater mussel, as a potential sentinel in determining freshwater microplastic pollution in the South American Pantanal region. The MPs accumulated more in the gills (78%) than the gut (72%) of the bivalves. The average MPs size found in bivalves was around 17–88  $\mu\text{m}$ . The organisms eliminated from 0 to 3300 particles during the study [56]. Research work was conducted by Weber and his team to study the effect of microplastics and thermal stress on *Dreissena polymorpha*, a freshwater mussel. Throughout the study, no significant interaction was observed between polystyrene microplastics and thermal stressors. However, thermal stress had a greater impact on mussels, which reveals their highly sensitive stress to temperature. In this study, dreissenid mussels did not react much to MPs in a controlled environment, but that might not be the case in an outside environment that is more polluted due to anthropogenic activity [57].

A study by Kolenda was conducted in Southwest Poland from May to June in 2017 and 2018 to analyze microplastic ingestion in tadpoles. A total of 201 tadpoles were collected from eight ponds. The tadpoles belonged to belonging to five different species. The particles were fibers majorly (69 items – 97%) followed by fragments (2 items – 3%). The mean length of fibers reached 2.2 mm. Diverse colors of MPs were seen. Spectroscopy revealed that all MPs particles had an anthropogenic origin and included a majority of nylon (90%), followed by polyisoprene (6%), polyurethane (2%), and 1,2-polybutadiene (2%). This study confirmed that pond-breeding amphibians are exposed to MPs [58].

A study that focused on estuarine gastropods was conducted by Zaki along the Klang River estuary. Ninety-five gastropod samples were collected from 12 sampling sites. All the samples had microplastic present in them. The MPs size ranged from 0.50 to 1.75 particles/g of wet weight tissue or 0.25 to 0.88 particles/individual. The mean MPs sizes ranged from 30 to 1850  $\mu\text{m}$  with a mean size of 538.30  $\mu\text{m}$ . Black was the most dominant MPs color observed in the samples. Fourier-transform infrared (FTIR) spectroscopy confirmed the presence of three different polymers: Polyethylene-propylene-diene (PEPDM) (48%), polyester (27%), and polyurethane (3%) [59].

#### 5. MICROPLASTIC STUDIES ON ZEBRAFISH

A study found that polystyrene microplastics increased the activities of catalase and SOD in *Danio rerio*. It also revealed that treatment with microplastics induced oxidative stress [60]. A study conducted by Lili Lei on Zebrafish *Danio rerio*, demonstrated that ingestion of microplastic particles leads to intestinal damage. Further, histological analysis revealed cracking of villi and splitting of enterocytes [61]. *Danio rerio* when exposed to naturally aged polystyrene microplastics at the intermediate development stage showed a considerable increase in parameters related to oxidative stress [62]. A unique study to check the transgenerational effect of MPs on the fish, *Danio rerio*, was conducted by Qiang. The polystyrene microspheres accumulated

in the intestines of the fish and showed critical fluctuations in mRNA expression levels; however, the offspring was unaffected, and the transgenerational effects were negligible even at high concentrations [63].

#### 6. UPTAKE OF MICROPLASTICS BY FRESHWATER FISHES

Microplastic pollution is majorly influenced by anthropogenic activity. Studies on the influence of microplastics in aquatic organisms are constantly on the rise. Di Sun, in 2020, researched to analyze and characterize the spatial distribution of microplastics in two economically valuable fishes, Tilapia and Mud Carp. Samples were collected from 25 sites of the north and west rivers of Guangdong province. It was found that 20/25 sampling sites had microplastics prevalence. In 76 samples of fish, a total of 160 MPs were found. In the samples, both of Tilapia (80%) and Mud Carp (77.8%) contained MPs. In Tilapia, MPs of three shapes such as pellets, fragments, and fibers were present, whereas, in Mud Carp, only fragments and fibers were found. Based on color, white was highly prevalent, and fragments were the most common shape of MPs found. In the samples, more than 74% of MPs were less than 1 mm in size, which suggests that the smaller the particle size, the higher the uptake of MPs [64].

In another study on riverine systems, the microplastics content in the brown trout *Salmo trutta* Linnaeus, 1758, was analyzed. A total of 58 fish from six sampling sites were collected for the study. A total of 92 microplastic particles were retrieved from the fishes, which consisted of 11 different polymers. Seventy-one particles were removed from the gastrointestinal tracts (GITs) contributing to 77% of total MPs accumulation. Fibers were the most common type of MPs, and the dominant size range was 350  $\mu\text{m}$  to 5 mm. The fish *S. trutta* is of good economic and commercial value. As confirmed by the study, the presence of MPs in them is a drawback and a negative trait for consumption [65].

The research was conducted to assess microplastic ingestion by the freshwater fish *Hoplosternum littorale*, which is economically and commercially important as it is heavily consumed in the semi-arid regions of South America. A total of 48 fishes were used for the analysis (40 males and eight females). Plastic debris was found inside the guts of 40 individuals, which amounted to 83% of the total sample. A total of 176 plastic particles were retrieved from the fishes, and the number of MPs particles ranged between 1 and 24 particles per fish. About 88.6% of the particles present in the fish gut were <5 mm (156 out of 176 particles). Fibers were the most prevalent type of MP recovered from the gut amounting to 46.6% in total. The study suggests that the gut content of fishes can be used as a qualitative tool to assess microplastic pollution in freshwater [66].

In an investigation carried out in the lower Xingu River Basin in the Amazon, 172 specimens of 16 *Serrasalminae* species were collected. In the samples, about 80% of species analyzed, and one-quarter of sample specimens had ingested plastic particles in the size range of 1–15 mm in length [67].

A study was conducted to analyze the level of plastic ingestion by the commercially valuable, planktivorous fish *Alburnus tarichi*. A total of 3338 pieces of plastic were collected from the GITs of the 101 sampled fishes. The abundance of MPs ranged from 8 to 124 pieces per fish with an average of  $34 \pm 13$  MPs/individual. Fibers constituted the samples majorly – 74%, and blue color MPS was highly prevalent (58%). Out

| Title of the research   | Author                 | Year | Major objective  | Innovative ideas, methods in use, strategies, and proposed solutions  | Reference |
|---|------------------------|------|--|---|-----------|
| The effects of plastic pollution on aquatic wildlife: Current situations and future solutions   | Sigler <i>et al.</i>   | 2014 | Plastic pollution management measures                                      | 1. Lagrangian drifters to monitor the trajectory of floating marine debris.<br>2. The Marine Debris Tracker – a citizen science project, which helps the community report plastic debris in the coastlines, and waterways from their smartphones.<br>3. Thermal degradation is the new way to recycle plastic waste   | [73]      |
| Microplastics in the marine environment: Sources, consequences, and solutions   | Thompson <i>et al.</i> | 2015 | Photo-oxidative degradation  | End of life plastics can be used as raw materials for new production.   | [74]      |
| Solutions to microplastic pollution – Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies | Talvitie <i>et al.</i> | 2017 | Wastewater effluent treatment  | 1. Membrane bioreactor for treatment of primary wastewater effluents<br>2. Tertiary treatment technologies such as disc filter, rapid sand filtration, and dissolved air flotation for secondary effluents treatment.   | [31]      |
| Microplastics pollution and reduction strategies  | Wu <i>et al.</i>       | 2017 | Microbial biodegradation, and bioremediation of plastic pollutants         | 1. Removing microbeads from personal care products<br>2. Usage of biodegradable products.<br>3. Reduce plastic usage, promote reuse, and improve recycling techniques<br>4. Improve the separation efficiency of wastewater treatment plants.<br>5. Development of clean-up and bioremediation technologies   | [75]      |
| Degradation of plastics and plastic-degrading bacteria in cold marine habitats  | Aneta <i>et al.</i>    | 2018 | Microorganism on marine water  | 1. Biodegradation of plastics using marine bacteria as potential organisms<br>2. Energy recovery and molecular redesign to be considered as well in addition reduce, reuse, and recycle.<br>3. Development of new bioplastic material is required   | [76]      |
| Solutions and integrated strategies for the control and mitigation of plastic and microplastic pollution                                | Prata <i>et al.</i>    | 2019 | Integrated measures for plastic pollution                                  | Life cycle assessment as a tool to improve plastic production   | [77]      |
| A novel method enabling the accurate quantification of microplastics in the water column of deep ocean                                  | Lie <i>et al.</i>      | 2019 | Novel filtration method for sampling microplastics                         | <i>In situ</i> filtration technology a novel sampling method for filtration of microplastics in the water column of deep ocean  | [78]      |
| Biodegradation and catalytic-chemical degradation strategies to mitigate microplastic pollution   | Zhou <i>et al.</i>     | 2021 | Catalytic-chemical degradation   | Biodegradation or catalytic-chemical degradation for degrading microplastics  | [79]      |
| Characterization and identification of microplastics using Raman spectroscopy coupled with multivariate analysis                        | Jin <i>et al.</i>      | 2022 | Spectral analysis for characterization and identification of microplastics | 1. Raman spectroscopy coupled with multivariate analysis as a robust analytical method to investigate the spectral profile of microplastics.<br>2. Support vector machine (SVM) classification achieved over 98% accuracy for polypropylene, polyethylene terephthalate, polyvinyl chloride, polycarbonate, polyamide, and over 70% accuracy for high-density polyethylene and low-density polyethylene | [80]      |

of five samples subjected to FTIR, three were polyethylene and two were polypropylene [68].

A study on the Japanese medaka (*Oryzias latipes*) juveniles based on exposure and depuration tests of fluorescently labeled MPs: Polyethylene (sphere with 20 µm or 200 µm diameter) and polystyrene (sphere with 2 µm or 20 µm diameter) revealed that MPs accumulated majorly in the

GITs of the medaka during the exposure study. In the fishes exposed to 20 µm PS- and PE-MPs, fluorescent signals of MPs were observed in the gills and head. The average bioconcentration factor for medaka at various concentrations is as follows: 200 µm PE-MPs – 74.4, 20 µm PE-MPs – 25.7, 20 µm PS-MPs – 16.8, and 2 µm PS-MPs – 139.9. The 2 µm PS-MPs had a higher uptake rate and lower depuration rate [69].

A study was conducted to determine microplastic contamination in the fish *Gambusia affinis*, which is an excellent freshwater environmental control agent. Microplastics <0.1 mm were prevalent in water samples, gills, and the digestive tracts of the fishes. Black color microplastics were the most dominant in the samples; water (24%), gill (43%), and digestive tract (46%). Fragments were the most abundant microplastic type. The water samples consisted of 4066.67 particles/m<sup>3</sup>, gill samples contained 1352.78 particles/gram, and 2138.89 particles/gram were present in the digestive tract. Multivariate analysis and *post hoc* test revealed a significant value below 0.05 ( $P < 0.05$ ), indicating that the abundance of microplastics varies between organ, water samples, and locations [70].

A study was done to assess the presence of microplastics in commercially sold fishes in Kerala. Both the edible and inedible tissues of pelagic fishes were taken into account. Samples of nine pelagic fishes were collected over a period of 6 months from December 2017 to May 2018. The average abundance of MPs in the edible tissues of fishes was  $0.07 \pm 0.26$  items/fish, and in inedible tissue, it was  $0.53 \pm 0.77$  items/fish. Fragments were the most common type of MPs found both in the edible (57.8%) and inedible (55.6%) tissue samples followed by fibers and sheets. Majority of MPs segregated were white in color. The microplastics were largely in the size range of 200–400  $\mu\text{m}$ , and polyethylene was the predominant polymer type [71].

## 7. CONCLUSION

More studies on microplastic pollution in freshwater resources are required. Studies on the negative impact of consuming fishes reared in a microplastic polluted environment are the need of the hour. Microplastics are abundant in the environment, and it is high time we realize the complexity of the issue. It is vital to evaluate the ways through which microplastics gain entry into the freshwater systems. In a review by Akdogan and Guven, more than 200 papers published between 2006 and 2018, were analyzed and they concluded stating “whilst marine microplastics have received substantial scientific research, the extent of microplastic pollution in continental environments, such as rivers, lakes, soil and air, and environmental interactions, remains poorly understood [72].” It is also pertinent to analyze the various modes in which aquatic organisms take up microplastics. We are now aware of the fact of microplastics presence in human blood. Studying model organisms and checking ways through which MPs travel along the food chain will help us better understand the current status of microplastic pollution, and come up with sustainable solutions.

## 8. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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## 10. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## 11. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

## 12. DATA AVAILABILITY

No data has been available for the present review.

## 13. PUBLISHER'S NOTE

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