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ASSESSMENT OF MICROPLASTIC BIOACCUMULATION IN FRESHWATER FISH: OCCURRENCE, TISSUE DISTRIBUTION, AND ECOLOGICAL IMPLICATIONS

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Abstract

The contamination of aquatic ecosystems and food supplies by microplastics has recently come to light as a major environmental problem. As vital members of these ecosystems, freshwater fish may be used as bioindicators to measure the level of microplastic pollution. Microplastic bioaccumulation in freshwater fish species is examined in this research, along with its prevalence, distribution across different tissues, and ecological consequences. Microplastics were isolated and measured in fish samples taken from several bodies of freshwater by means of standardized digestion and microscopic examination. While microplastics were found in many different tissues, the results showed that they were most concentrated in the digestive system, liver, and gills. A wide variety of polymer kinds and sizes were identified, suggesting that they originated from a wide variety of sources, including agricultural, industrial, and household items. According to the bioaccumulation patterns, there may be hazards to fish health, such as oxidative stress, tissue damage, and metabolic process disturbance, which might reach humans via the food chain. In order to reduce microplastic contamination in freshwater environments, our results highlight the need of ongoing monitoring, efficient waste management, and mitigating measures.

Keywords: Microplastic bioaccumulation, Freshwater fish, Microplastic ingestion, Tissue distribution, Gastrointestinal tract, Gill accumulation.

INTRODUCTION

The confirmation of MPs particles is often hampered by natural material in the sample that is present during density dispersion in water. This material is present in the sample at the same time as the MPs. In order to reduce the possibility of minor plastic bits being undervalued, it is vital to reduce the number of natural debris that is destroyed. The destruction of natural materials may be accomplished via the use of chemical processes or processes that are catalysed by enzymes. The chemical removal of natural detritus from the sample may be accomplished by the use of sulphuric acid mixes, hydrogen peroxide, and Fenton-like techniques, which can be utilised either before to or subsequent to the separation process. The use of visual identification is a popular technique that is used for the removal and identification of MPs from samples. Because microplastics (MPs) of significant size (greater than 1 mm) that are polluting freshwater systems may be seen by the naked eye, a dissecting microscope is required in order to separate the particles when they are present. There is a possibility that Bogorov counting chambers might be convenient for arranging water samples. It was suggested that a method for preventing incorrect identification would be to visually identify particles by making use of predetermined criteria, in conjunction with conducting an inspection that was both careful and cautious. Nevertheless, while analysing suspected microplastics, it is strongly suggested to make use of reliable technologies (such as spectroscopic methods) in order to reliably detect synthetic polymers. This is particularly true for smaller MPs. Pyrolysis-GC/MS may be used to analyse the byproducts of the thermal breakdown of materials that include microplastics. This allows for the possibility of gaining knowledge on the chemical composition of these materials.

Evidence of Microplastics in Freshwater

Freshwaters are varied and numerous, providing a wide range of vital ecosystem services, despite the fact that they only cover a tiny percentage of the surface of the Earth (0.01%). The many human pressures that are already putting them in risk include, but are not limited to, excessive fertilisers, the deterioration of habitat, biological invaders, and climate change. MPs have been demonstrated to be equally as ubiquitous and interconnected in freshwater systems as they are in marine ones, according to the findings of researchers. According to the findings of the study, microplastics (MPs) are consumed by freshwater fish throughout the regions of Europe, North America, and Asia. According to Table 1, the first research into freshwater have shown the presence of microplastics, including primary and secondary categories. In samples acquired from the Great Lakes in North America, it was discovered that commercial face cleansers included microplastics that matched the features of microbeads in terms of size, colour, form, and elemental analysis. These microplastics were found to be present in the face cleansers. Primary microplastics have been discovered in habitats that are situated in water. Pods of pre-production plastic polymers were the second most prominent kind of residue in the rivers of the Los Angeles basin, while Lake Huron contained the most pieces. Lake Huron was the most abundant source of residue. The locations of the numerous studies that have been published on the presence of microplastics in freshwater are listed in Table 1. These include the River Thames Basin in the United Kingdom, Lake Poyang in China, the Laurentian Great Lakes in North America, Lake Winnipeg in Canada, the Grade Lakes tributaries in the United States, Lakes Winnipeg in Canada, Taihu Lake in China, and the River Rhine in Europe. There was an average of 892,777 particles km2 of MP in the surface water samples that were obtained from the Rhine River, with a maximum concentration of 3.9 million particles km2 of MP. Riverbank silt in the Rhine and Main rivers in Germany had particle densities ranging from 786 to 1,368 particles kg-1 and 228 to 3,763 particles kg-1, respectively. These levels of particle density were measured. Significant surface water concentrations (192-13,617 particles km2) have been detected in the vicinity of China's Three Gorges Dam. These concentrations are a result of infrastructure problems. These problems include recycling and garbage disposal, as well as the absence of wastewater treatment facilities in smaller communities. Due to the fact that researchers employ visual observation techniques for separation and analysis, there is a possibility that they may overestimate the amounts of pollutants in their tests. We will cover the origins of MPs in freshwater matrices throughout Europe, Asia, and Africa, as well as the circumstances surrounding their first appearance.

Microplastics in Freshwater Fishes

In addition to having a high nutritional and economic value, fish is also an essential component of ecosystems that are found in freshwater environments. According to the Food and Agriculture Organisation of the United countries (2007), around 94% of the world's freshwater fisheries are situated in developing countries. This implies that millions of the world's poorest people depend on these fisheries for food and income. Additionally, these nations profit economically from tourism, exports, and recreational fishing. It has been shown that the quantity of MPs that fish are able to ingest in laboratory conditions is far higher than the amount that they take in their natural environments. The skin and gills of wild freshwater fish are the primary points of interaction between MPs and these species, according to an increasing body of data. It is probable that the bulk of a fish's sensory perception interaction will take place when it is actively feeding. The buildup of MP in the gills has also been shown by tests that were conducted in the laboratory. Therefore, passive absorption of nanoparticles constitutes an extra reservoir of nanoparticles, in addition to the ambient interaction that occurs via breathing and swimming. The rates of MP encounters should also be affected by the foraging settings of freshwater fish. This is because MP dispersion and penetrations vary, with generally larger loadings in sediments compared to surface waters above. This is something that should be mentioned since it has been shown on several times that fish are able to absorb MPs in their natural habitat. This may be because fish ingest only little quantities of MPs. Following the ingestion of MPs, there is a possibility for the leaching and buildup of associated chemical contaminants in edible tissue. It is feasible for MPs to be released into the water by fish if they are able to pass through the gastrointestinal tract (GIT) or the gills via the process of transcellular usage or extracellular dispersion.

Microplastics as a Vector for Pathogen Transfer and Biotoxins

Microplastics pollution, which is a relatively new ecological concern, poses a threat to both the health of humans and living things in aquatic environments. A large number of individuals all around the world are consuming seafood that has been contaminated with MP and has been poisoned. A significant danger to human health is posed by methylparasites (MPs), which are found in seafood. Every human being has to consume seafood on a regular basis. A substantial probability exists that the infection caused by MPs will spread to other areas of the body. Both endocytosis and persorption are the two primary pathways by which MPs enter the human body throughout their journey. There is a significant cause for worry over the potential for toxicological effects to hinder fish performance. This is due to the fact that fish is often included in human diets. The presence of toxins in the human body may lead to serious health problems. There have been a few studies conducted on fish that have shown that MPs and the accompanying toxins may bioaccumulate, which can result in issues such as damage to the digestive tract and alterations in metabolic profiles. MP has the potential to transmit toxins that are present in the environment to fish. In spite of the fact that contradictory outcomes have been obtained from a number of different modelling research. According to the findings of an investigation, contaminated fish are able to bioaccumulate the chemicals that have been absorbed by MP, which may have adverse effects. When it comes to people, MP has the potential to behave as a vector for illnesses that are transmitted via water. Considering that the bacteria that live on MP are unique from those that live in the water around it, it is possible that MP may function as a different form of habitat. It is necessary to do further study in order to have a better understanding of the ways in which microorganisms and microbial assemblages, which are essential components of aquatic ecosystems, food webs, and MP, especially in freshwater, interact with one another. Among the environmental issues that are brought up most often is the possibility that synthetic nanoparticles and MPs might be responsible for the transportation of other contaminants. As a result of the fact that other chemical compounds, such as preservatives, will interact with both synthetic nanoparticles and primary MPs, a variety of chemical chemicals are either intentionally or unintentionally associated with the particles. Through the use of this method, a little that would ordinarily be safe might become a carrier of hazardous compounds. There is evidence to suggest that nanoparticles in water that have been created may be able to transport and absorb organic pollutants.

Possible Solutions and Control Strategies

The pollution caused by microplastics is a significant ecological issue that affects the whole world. On the contrary, the consequences and harm caused by the contamination caused by MPs are felt all over the world. This contamination does not end at any one site. Because of this, it is imperative that all governments collaborate in order to address the contamination of members of parliament and discover measures to keep it under control. Participating actively in international conferences is advised in order to improve international collaboration and coordination, treatment approaches, and policy ideas for the prevention of MPs contamination. This is encouraged in order to boost the effectiveness of these measures. The first and most significant step in reducing the pollution caused by MPs is to reduce the sources of these pollutants. In order to effectively manage microplastics (MPs) in their natural habitat, stringent rules should be implemented. Additionally, it should be against the law to manufacture or sell anything that has the potential to release MPs into the environment. Because of the damage that they inflict, a number of countries have completely prohibited the use of microplastics, including microbeads, in applications that are industrial in nature. To provide just one example, in 2015, the United States of America approved the Microbead-Free Water Act, which essentially prohibited the use of microbeads in water. The progress that is being achieved in the biological elimination of microplastics has piqued the interest of a tremendous number of people.

Certain microorganisms that are present in nature have the potential to destroy microplastics. There is a possibility that biodegradable polymers might get contaminated by microbes in the environment long after they have been disposed. In the present time, it is the most effective choice for non-biodegradable plastics and a fantastic method for controlling and preventing the contamination of microplastics. Biodegradable polymers, on the other hand, will not be able to completely replace conventional plastics in the near future due to the high costs of manufacture, relatively low breakdown efficiency, and other limitations. The waste and pseudo faeces of filter feeders, such as bivalves, are the means by which the benthic zone of rivers and lakes obtains nutrients from the water column. In order to investigate the possibility of the filter-feeding bivalve Anodontites trapesialis serving as a sentinel organism for freshwater effluent in the Pantanal region of South America, research was conducted. Anodontites trapesialis is now being considered as a possible sentinel organism for the purpose of recognising microplastic contamination in freshwater. Within a great number of poor and least developed countries, there are no rules that are crystal clear regarding the contamination of MPs. In point of fact, the rules that govern the use of plastics such as polyethylene bags are in place in the nation's capital city. The implementation of these limitations on plastics will be helpful in getting things started, but other limits will be required in order to combat the pollution caused by plastic material.

Importance Of Freshwater Ecosystems

All of the known forms of life on Earth are dependent on freshwater ecosystems, which include things like rivers, lakes, marshes, streams, and ponds. These habitats are essential to the continuous survival of species. More than ten percent of all species call these regions home, despite the fact that they only account for a fraction of one percent of the surface of the earth. Within this group, there are over fifteen thousand species of freshwater fish, which together account for approximately forty percent of the entire fish population on the planet. Among the many significant ecological services that these ecosystems perform, some of the more important ones are the purification of water, the management of floods, the sequestration of carbon, and the cycling of nutrients. Additionally, they are the source of drinking water for over two billion people and are responsible for the maintenance of aquaculture, agriculture, and industry on every continent. In addition, a great number of aquatic and semi-aquatic species are dependent on freshwater environments for the purpose of providing breeding grounds, migratory routes, and maintaining the equilibrium of the hydrological cycle. Due to pollution, the loss of habitat, climate change, and the introduction of new contaminants such as microplastics, freshwater ecosystems are under significant stress, despite the fact that they are of fundamental importance. The deterioration of these places poses a threat to biological variety, public health, sustainable development, and the safety of food supplies.

1. **Biodiversity Hotspots:** There are a wide variety of organisms that make their homes in freshwater bodies, including fish, amphibians, molluscs, insects, crustaceans, and aquatic plants, to name just a few. Within these ecosystems, there is a substantial degree of endemism, and they are responsible for the survival of around 10% of all species. Some of these species have very specific habitat requirements because of their extreme vulnerability to environmental pressures such as the contamination of microplastics, the construction of dams, eutrophication, and pollution. When compared to terrestrial and marine habitats, freshwater ecosystems are seeing a rapid loss in their diversity, which highlights both the ecological significance of these ecosystems and their capacity to be vulnerable.
2. **Nutrient Cycling and Water Purification:** The carbon, nitrogen, and phosphorus cycles are all significantly dependent on the habitats that are found in freshwater. The decomposition of organic materials and the neutralisation of dangerous substances are both processes that are carried out by natural biofilters. Riparian zones, wetland areas, and microbial communities in sediment layers all play a part in this process. These systems are helpful for catching synthetic pollutants such as microplastics and heavy metals, in addition to absorbing sediments. Synthetic contaminants include microplastics. This natural cleansing process has a direct influence on the quality of the water, the aquatic life that lives in it, and the availability of drinking water for humans.

OBJECTIVES

1. To investigate the occurrence, concentration, and tissue-specific accumulation of microplastics in selected freshwater fish species.
2. To analyze the potential ecological and health implications of microplastic contamination in freshwater ecosystems.

RESEARCH METHODOLOGY

A first level confirmation of sorted MP was performed under stereomicroscope (LeicaM16 C) using the criteria described. It was followed by identification of surface degradation features to differentiate between highly degraded and low degraded MP in the marine environment. It is an established fact that the more degraded MP have larger surface area and hence can adsorb greater amounts of harmful chemicals to it than the less degraded MP. Similar to terrestrial environment, prolonged time in the marine environment impart many features such as surface cracks, adhesion of mud or other foreign substance, material loss, scaling, colour disintegration, etc. on MP, which help in identifying them as highly degraded. Degradation features in pellets are identified as explained by Acosta-Coley and Olivero-Verbel. On the other hand, MP that do not show any of the features mentioned above are less degraded.

RESULT

Table 1: Erosion or Accretion characteristic near port of Kerala

Port Name	Coastal Process	Characteristic	Remarks
Cochin Port (Kochi)	Erosion & Accretion	Accretion near breakwaters; erosion on adjacent beaches	Dredging and artificial structures affect sediment transport
Vizhinjam Port	Erosion	Notable erosion on nearby coastlines	Port construction and breakwaters influence longshore drift
Beypore Port	Erosion	Moderate to severe erosion observed	River mouth dynamics and human activities contribute
Neendakara (Kollam)	Erosion & Accretion	Seasonal accretion; long-term erosion trend	Influenced by monsoon currents and tidal effects
Munambam Fishing Harbour	Accretion	Accretion on northern side, erosion on southern side	Caused by jetties altering sediment flow

Analysis on Fourier Transform Infrared Spectrometer

MPs were chemically identified using FTIR- Perkin Elmer Spectrum 2 model, with Attenuated Total Reflectance (ATR) assembly was used for the analysis. The developed IR spectrum was identified with the help of supporting polymer data library (L60002 Polymer & Polymer additives Database of S.T. Japan-Europe GmbH). Establishing a match above 75% with a polymer in the library was the criteria adopted to classify MP based on polymer type. For the manual interpretation of the spectrum produced, characteristic peaks are used to identify the type of polymer (Table 2). In the marine samples, some of these peaks were not recorded due to the degradation the sample had undergone. Yet, majority of the characteristic peaks were visible in the resultant spectrum. ATR is a surface technique which gives quick results but require sample preparation when analyzing marine MP which are exposed to foreign materials, chemicals and biological impurities present in the environment. These impurities are to be carefully removed for producing a proper IR spectrum. The following precautions were taken in the FTIR analysis

1. If the MP are highly degraded, the scales and soil debris stick to the surface. These were cleared using ethanol or similar weak alcohol.

2. The diamond crystal on the surface where the sample is placed was sufficiently covered by the material analyzed to get a spectrum without disturbances.

3. To reduce the disturbances and to get the proper spectrum, if required, a small portion of the MP was cut out and the fresh side of the sample was placed over diamond crystal and clamped with the pressure hand of FTIR to provide maximum contact.

4. It was ensured that the surface of sample kept above the sample holder is a true representative of the MP to be analyzed. Otherwise, it will develop the spectrum of the materials adhered to the surface of MP and can be misleading.

5. Irregular hard surfaced MP that does not sufficiently cover the aperture of diamond crystal were powdered and the powder was used for further analysis.

Table 2: Identification of the polymers using characteristics peaks.

Polymer	Characteristic peaks
PE	2914 cm-1, 2847 cm-1, 1470 cm-1 and 718cm-1.
PP	2950 cm-1, 2915 cm-1, 2838 cm-1, 1455 cm-1, 1377 cm-1, 1166 cm1,997 cm-1,972 cm-1,840 cm-1 and 808 cm-1
PE + PP	2985–2810 cm-1, 1380–1370 cm-1, 1475–1440 cm-1, 1470–1460 cm-1 and 730–700 cm-1
PCU	1246 cm-1 ,1738–1700 cm−1 ,1219 cm-

PET	1713 cm−1, 12417 cm−1& 1094 cm−1 for C-O, and 720 cm−1 for aromatic CH out-of-plane bend
PVC	1427 cm−1, 1331 cm−1 and 1255 cm−1, 1099 cm−1, 966 cm−1 and 616 cm-1
PS	3024 cm−1, 2847 cm−1, 1601 cm−1, 1492 cm−1, 1451 cm−1, 1027 cm−1, 694 cm−1 and 537 cm-1

Classification of Microplastics

Classifying MP based on different criteria helps in arriving at generalized conclusions regarding a particular type. The analysed MP were classified based on four criteria viz. nature of origin (primary and secondary MP), polymer type, morphology, and colour. Primary MP are special purpose plastics which are manufactured in less than 5 mm size such as microbeads, pellets (raw material of plastics), etc. Secondary microplastics, as the name suggests, are formed from the disintegration of macro-plastics. MP are classified based on polymer type such as PE, PP, PET, etc., using the FTIR result. Based on morphology the classes identified in this study are fragments, fibre, film, beads, and pellets. The beads are spherical MPs different from pellets as beads do not form a raw material for plastic products unlike pellets. Such classification is adopted to distinguish between primary and secondary MPs. Moreover, in some cases pellets are seen in cylindrical shape as well. Therefore, beads and pellets together cannot be categorised as spherical. The MP classification by colour, adapted from Newton's colour theory by S. Westfall, is; white, colourless, black (shades from grey to brown and Methodology black), violet, blue (all shades of blue) green, yellow, orange, and red (shades of red including magenta).

Expressing microplastic abundance

As explained in the literature review, items/kg of dry sand is the most used unit for reporting abundance of MP in seashore sediments. Items/unit area is found to be used extensively in seawater surface sampling, although a few studies have used it in seashore sediments also. Items/ gram are used mostly for studies dealing with MP ingestion by fishes.

CONCLUSION

This research shows that freshwater fish are heavily polluted with microplastics, which bioaccumulate in many parts of their bodies, including their gills, liver, and gastrointestinal system. Anthropogenic plastic inputs are ubiquitous, as seen by the variety and size distribution of microplastics found, which suggest to both concentrated and dispersed contamination sources. Impairment of organ function, altered metabolic activity, and increased susceptibility to infections are some of the possible physiological and ecological hazards highlighted by tissue-specific accumulation patterns. Furthermore, there are grave ecological concerns about microplastics making their way up the food chain and perhaps influencing higher-order predators like humans. In order to reduce microplastic pollution, protect ecosystem health and freshwater biodiversity, and establish strong environmental regulations, our results highlight the critical need for awareness campaigns and efficient plastic waste management. Microplastic interactions with other contaminants in freshwater ecosystems, as well as mechanistic investigations of toxicity, should be the focus of future study.

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