DETERMINATION OF MICROPLASTIC IN SELECTED FRESHWATER FISH SPECIES FROM AGRICULTURE FISHPOND IN TANJONG KARANG, SELANGOR, MALAYSIA

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Abstract

As the amount of plastic in the environment continues to rise, one of the most significant environmental challenges that has emerged is the contamination of seafood with microplastics (MPs). This study was conducted in Tanjong Karang, Selangor, in order to investigate the prevalence of MPs, their properties, and the range of variation among them in different species of freshwater fish. Twelve different fish species from a variety of feeding zones were investigated to establish the degree to which MPs consumption rates differed from zone to zone. It was found that MPs were present in the gastrointestinal tracts (GIT) of 29.2% of the fish samples, which is a higher percentage than what has been found in other regions that have been documented. When compared to the other fish species, the *Anabas testudineus* has the greatest concentration of MPs. Examinations at a microscopic level indicated that the film morphotype and the colour white were predominant among MPs. Fourier Transform Infrared analysis showed that the fish gastrointestinal tract contained high-density polyethene and polypropylene as the predominant polymers. Plastic ingestion in fish may be related to the feeding zones, as indicated by the fact that demersal fish have a greater concentration of MPs compared to benthopelagic and pelagic fish. This finding will help people have a better understanding of which types of freshwater fish and which types of feeding zones contain greater levels of MP contamination.

Keywords: Microplastics, Freshwater Fish, Feeding Zones, Feeding Habitats

Introduction

Microplastics (MPs) are plastic materials which above 0.1 micrometers to 5 micrometers in dimension. In comparison, nano plastics are sized less than 0.1 micrometers (1). Since 1950, the production of plastic materials has increased by a factor of 245, which means that there are presently 388 million tonnes of plastic materials across the globe (2). Frequent occurrence of MPs in marine ecosystems as emerging contaminants have attracted widespread concern, in freshwater ecosystems and terrestrial ecosystems (3).

As a result of the region's rapid economic and population development, as well as its increasing urbanization, Asia is regarded as a major source of plastic pollution (4). Malaysia is one of the Asian countries included in the hotspot. Malaysia is one of the top 20 countries in terms of the amount of plastic waste that it sends into the ocean each year, with an emission rate of 7.3 X 104 metric tonnes (5). No legislation or restrictions are limiting the emissions of MPs into the environment, particularly water bodies such as lakes and oceans, even under the Environmental Quality Act of 1974 (EQA). The Malaysian government has long underestimated the importance of eliminating MPs contamination in the environment. This situation occurs because the federal government lacks the urgency and commitment to implement regulations and guidelines regulating the emission of MPs into the environment, due to lack of understanding and information about the impacts of MPs on human health (6).

Despite the fact that MPs in freshwater fish at the aquaculture farm pose a significant danger to marine life and human welfare, little research has been conducted (7). According to Lam et al. (2), MPs ingestion by cultured fish receives less attention than that by wild fish because previous research concentrated on the presence and distribution of microplastics in agricultural soils. To date, the data available in this field is very scarce.

By consuming fish containing MPs, humans will be exposed to toxic substances at multiple trophic levels. Moreover, fish and other organisms that consume MPs may be exposed to pollutants in the polluted surrounding environment, which may bioaccumulate and biomagnify (2). The fish is also affected, fish mortality increases, and food security is threatened. Therefore, plastic pollution is a significant concern that necessitates research together with immediate and long- term actions to reduce ecological, social, and economic harmful effects (2).

The main goal of this research was to identify the characteristics and abundance of MPs in various fish species with varying feeding zones: near surface of water (pelagic), midwater (benthopelagic), and deep water (demersal).

Materials and Methods

Sample collection

The study was carried out at one fishpond station at the boundaries of the rice fields in Tanjong Karang, Selangor. Four species of fish (*Siluriformes sp., Oreochromis niloticus, Leptobarbus hoevenei* and *Anabas testudineus)* were collected regularly in triplicate (Figure 1). Fish samples were stored in an icebox before being transported to the laboratory at the Faculty of Health Science in UiTM Puncak Alam, Selangor, and stored in the freezer at −20 °C (8). Fish sampling was treated based on the method by Free et al. (9).

The fish samples were brought into the laboratory, allowed to thaw to room temperature, and then important biological characteristics such as length (cm) and weight (g) were measured. The gastrointestinal tract (GIT) of each sample was dissected, and its contents were collected in order from the oesophagus all the way to the end of the tract. After that, each sample was placed in its own individual container and stored in an ethanol solution that was 80% strength until it was analyzed further. Plastic materials were not utilized because it was anticipated that their use would result in cross-contamination as well as prejudice in the findings.

a) *Siluriformes sp.*

b) *Oreochromis niloticus*

c) *Leptobarbus hoevenei*

d) *Anabas testudineus*

Figure 1: Fish species collected from fishpond station at the boundaries of the rice fields in Tanjong Karang, Selangor

The alkaline technique was used to digest the sample. The GITs were placed in a glass beaker containing the digesting solution, 10 M sodium hydroxide (NaOH). Then, 50–60 g of digested samples were deposited in a water bath at 60°C and continuously shaken (130 rpm) during incubation to accelerate the digestion process. The sample is examined every seven days, and the digestive processes were replicated three times. The digestion fluid was filtered after 21 days.

After that, the samples were examined under a stereoscopic microscope (Optical microscope Leica DM2500) for microplastic components. A representative sample of microplastics (MPs) from each morphotype was randomly chosen for analysis using Fourier Transformed Infrared (FTIR) spectroscopy in order to identify and validate the properties of microplastics. The MPs were spread on a crystal of potassium bromide (KBr). The observations were taken in transmission mode at wavenumbers ranging from 400 to 4000 cm-1 (10). SpectraBase™ datasets from John Wiley & Sons, Inc., an online spectral repository, were used to identify the absorption bands of each polymer.

This study divided the observation of MP's characteristics in samples into three categories: MPs' morphotype, size, and color. Morphotype was based on a previous study by Li et al. (11). Morphotype can be fragment, flakes, fiber, pellet, film, and foam. Fragment is a complex and jagged plastic particle. Fibers are thin or fibrous and straight plastics. Pellet is complex and rounded plastic particles. Film is thin plane of flimsy plastics. Foam is lightweight and sponge-like plastic. Furthermore, based on a previous study by Lv et al.

(3), MPs size was divided into < 500 ***µm***, 500 ***µm*** – 1 mm, and 1 mm – 5mm. MPs color were recorded according to Jones et al. (12).

Data analysis

Kruskal-Wallis test was used to compare the abundance differences of microplastics in four different feeding habits of fish using Statistical Package for the Social Sciences (SPSS) version 28.0 where $p < 0.05$ were considered as statistically significant. Microsoft Excel was used to tabulate the characteristics of MPs.

Results

Microplastic abundance in fish

Throughout the investigation, 12 fish representing four species were captured and analyzed. The percentage of contaminated microplastic was calculated based on the previous study by Parvin et al. (10), and the result showed that 29.2% of the MPs were contaminated by 117 plastic particles found in the GIT of 12 different fish. During the observation, *Siluriformes sp.* had 25 particles, *Oreochromis Niloticus* had 30 particles, *Leptobarbus hoevani* had 28 particles, and *Anabas Testudienus* had 34 particles. Kruskal-Wallis test showed no significant differences in the abundance of MPs between the fish species (p = 0.760, p > 0.05). Figure 2 shows the highest microplastic abundance was found in the GIT of *A. testudineus* (34% plastics particles) followed by *O. niloticus* (30%), *L. hoeveni* (28%), and the lowest abundance in GIT was *Siluriformes sp.* (25%).

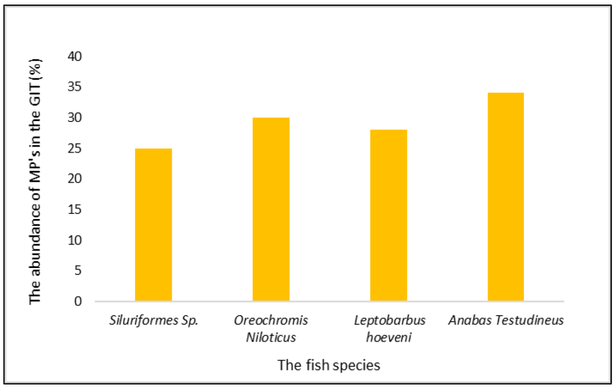


Figure 2: The abundance of MPs found in freshwater fish

The morphotype, color, and size of microplastics

MPs of various morphotypes, colors, and sizes were discovered in the fish sample in this research. Figure 3 shows the morphotypes of MPs show a different variety, such as foam, flakes, fibers, fragment, film, and pellets at difference fish species.

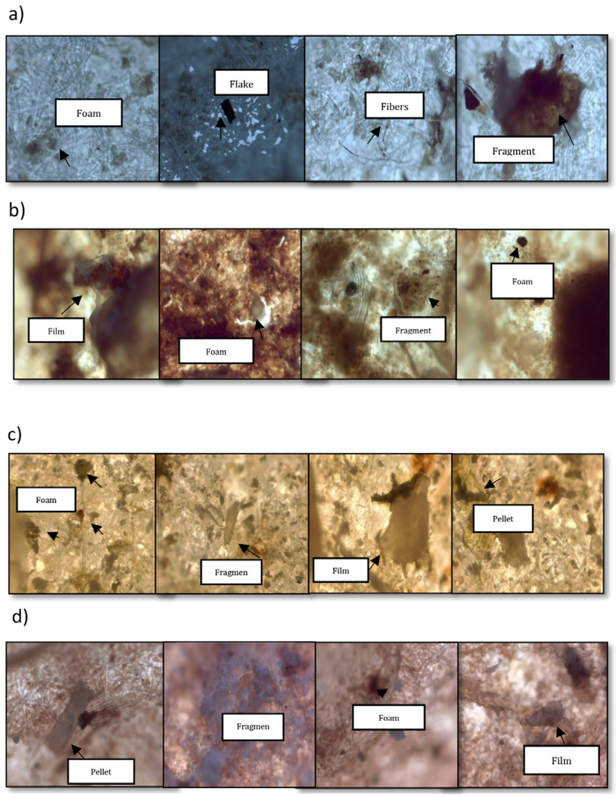


Figure 3: The different morphotypes of microplastics obtained from a) *Siluriformes sp.,* b) *O. niloticus*, c) *A. testudineus*, and d) *L. hoeveni*

Figure 4 shows the percentages of fragments, flakes, pellets, film, fibers, and foam observed in the GIT of fish. Film was generally the most dominant morphotype, accounting for 35% of the total MPs. Fragment made up 26% of the total, while pellet made up 22%. Meanwhile, flakes accounted for 10%, and the lowest morphotype was fibers which accounted for 2%.

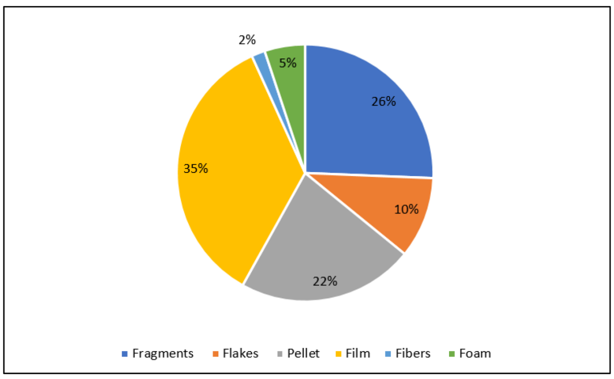


Figure 4: The percentage of MP's morphotypes

Furthermore, this research discovered six different colours of MPs in the GIT samples studied: transparent, green, yellow, brown, white, and black. *Siluriformes sp.* MPs come in six distinct colours: four brown, six white, five black, and five yellow. *Oreochromis niloticus* possesses five transparent, fifteen white, six black, and four yellow hues. Meanwhile, *Leptobarbus hoeveni* had five transparent, eight blue, two green, ten brown, and two white. *Anabas Testudineus* comes in 23 transparent, two green, fourteen yellow, twenty-three brown, thirty-three white, and fourteen black varieties. Figure 5 shows that white MPs were the most abundant at 28.2% which. The second highest color was transparent, accounting for 19.7%. Brown accounted for 18.8%, yellow and black accounted for 12.0% each, and the lowest was green, which accounted for 2.6%.

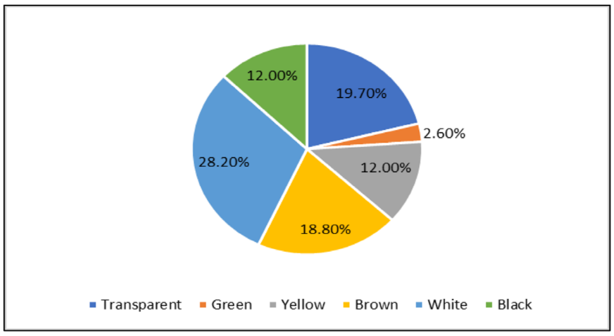


Figure 5: The percentage of MP's colors

Plastic particles in this research were divided into three sizes: 500 m, 500 m - 1 mm, and 1 mm - 5 mm. Based on Figure 6, there was 57.5% with size < 500 ***µm***, 22.5% with size 500 ***µm*** – 1 mm, and 20% with size 1mm – 5mm.

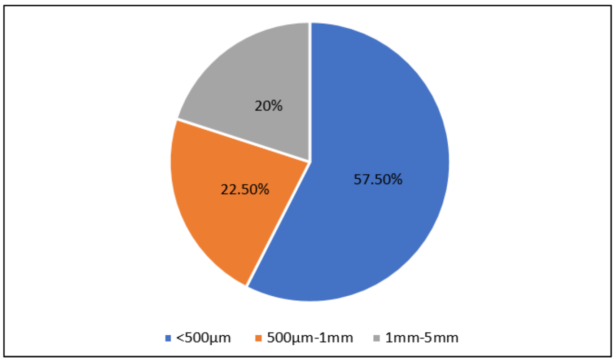


Figure 6: The percentage of MP's sizes

Microplastic's abundance and feeding zones

The feeding zone of *A. testudineus* was demersal (deep water), *Siluriformes sp.* and *L. hoeveni* were pelagic (near surface), and *O. niloticus* was benthopelagic (midwater). Figure 7 shows that the abundance in the demersal fish *A. testudineus* was the highest followed by pelagic fishes *Siluriformes sp* and *L. hoeveni.* The lowest was benthopelagic which is *O. niloticus*.

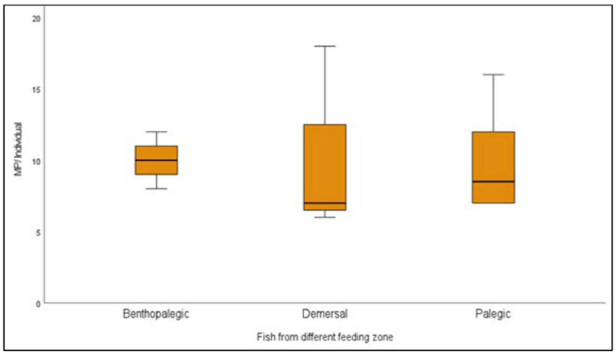
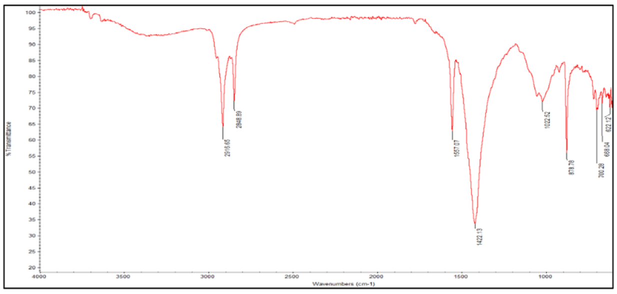


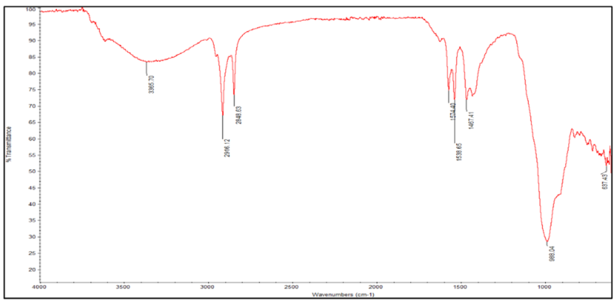
Figure 7: Comparisons of abundance of plastics among fishes from different feeding zones

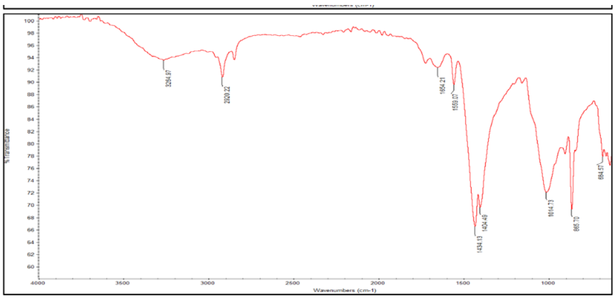
Chemical composition of microplastics

A significant number of samples (33.3%) from the 52 suspected MPs subsamples were verified by FTIR analysis. Polypropylene (PP) and PE polyethylene were recognised as polymer types using FTIR. Figure 8 (a) shows PP produces prominent peaks around wave number regions 3264.97 cm^{-1} (=C-H stretch), 2920.22 cm^{-1} (C-H stretch), 2916.65 cm^{-1} (C-H stretch), 878.78 cm^{-1} (C-H oop), 700.28 cm^{-1} (=C-H bend) and 668.04 cm^{-1} (=C-H bend). Figure 7 (b) also shows PE produces prominent peaks around wave number regions 1646.32 cm^{-1} (C=C-stretch) and 641.06 cm^{-1} (C=C-H; C-H bend). These results are consistent with previous efforts to determine the composition of PP and PE (13, 14).

a) Polypropylene (PP)







b) Polyethylene (PE)

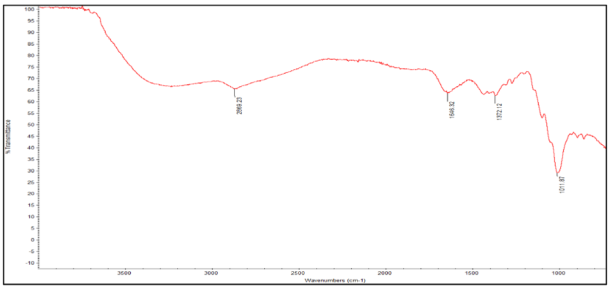


Figure 8: FTIR spectra of the representative microplastic found in freshwater fish sample a) PP and b) PE

Discussion

During the study, 12 fish from four different species were caught and tested. A total of 117 plastic particles were found in the digestive tract (GIT) of 12 different fish, which meant that 29.2% of the MPs were contaminated. The results can be compared to previous findings: in Bangladesh, 73.3% of 48 GIT fish are contaminated with MPs. (8); in Amazon River, 13.7% MPs of 14 fish species (15); and in the Gulf of Mexico just 4% (16). This is supported by Szymańska and Obolewski (17), spatial and temporal variations in the abundance and distribution of riverine MP are influenced by a number of parameters, such as flow velocity and proximity to MP sources within the catchment. In addition to this, freshwater matrices tend to be located close to point sources and in areas that have a substantial population. Urbanization is also a factor that contributes to an abundance of MPs (18).

Kruskal-Wallis analyses revealed no significant differences in MP abundance among the fish species ($p = 0.760$, $p > 0.05$). In a study of freshwater fish from the Han River, Kruskal-Wallis test revealed that there was no significant difference in abundance between species ($p = 5.548$, $p > 0.05$) (16), and in Thailand, there was no significant difference in the abundance of microplastics ingested by different species ($p = 0.849$, $p > 0.05$) (18).

Many factors such as fishing activities (15), laundry (19), net disintegration, and human activities (20) influence the abundance and distribution of MPs in the water. In this study, the fish were collected from the boundaries near the rice fields and homestays, where there are many sources of fish contamination, such as plastics from human activities, which were finally washed into bodies of water by wind and rain, fragmented, and ingested by fish. The sources of MPs in fish may come from the homestays, which includes cosmetics, cleansing agents, laundry, and facial scrubs (21).

In addition, organic fertilizers used in paddy fields were another factor that influenced the abundance of MPs. According to the findings of a previous research, organic fertilizers, which are frequently utilized in farming and gardening, tend to be an overlooked source of microplastics. This includes fertilizers that are prepared by composting and fermentation (22). The microplastics in wastewater and biosolids used for irrigation and fertilizing are also sources of abundance of MPs (23). In addition, the application of sewage sludge as fertilizer on agricultural soil was identified by a number of studies as possibly being a source of MPs. (3, 24, 25).

The different sources of MPs influenced the variety of morphotypes (17) and the fish's feeding zone also influences the morphotypes of MPs (20). The highest morphotype i.e., film came from the decomposition of plastic bags surrounding the pond, which the fish consumed. Fragment was the second most common morphotype, similar to a previous study (26). Secondary MPs were indicated by fragments and film because these are typically created by the breakdown or fragmentation of big plastic components (27).

The previous studies reported that secondary MPs are more abundant in the aquatic environment than primary MPs (28, 29). The presence of fibers in these experiments further demonstrated that the sources were secondary MPs, as fibers are typically found in clothing and other textile goods or by-products (30). Most of the MPs retrieved in this research were secondary, according to the MPs identified. These findings were in line with those of a research that was conducted in Bangladesh on fish and shrimp that came from the Bay of Bengal, which found that the secondary source was responsible for most of the MPs contamination and the formation of a morphotype (31).

From this study, white was the highest MPs color and followed by transparent. The white MPs were identified more frequently in fish stomachs than in water and sediment because they imitate the food sources of the fish (32). Regarding MPs color, the fishes were confused with their preferred prey and then ingested the MPs (33). It's not uncommon for shopping bags, packaging bags, textile fabrics, and even fishing nets to have some sort of colouring on them (34). Color is often a sign that there is more desire for and use of a wide range of plastic products in our daily lives. This has led to a huge amount of coloured plastic waste (35).

In this study, identified plastic particles were classified into three sizes, < 500 µm, 500 µm – 1 mm, and 1 mm – 5 mm. The classification of plastic particles followed a previous study from Bangladesh. The size of MPs in this study is not similar to the previous study that found microplastic sizes ranging from 1 to 5 mm as more abundant (36).

From this study, demersal fish was the highest abundance of MPs. The abundance's results and different habitats group can be compared to previous findings that the demersal fish consumed more MPs than pelagic and benthopelagic fish (37). However, Neves et al. (38) discovered that the MP content of pelagic fish species was higher than that of demersal fish species. On the other hand, recent studies reveal much larger abundances of MPs in demersal fish than in pelagic fish species (39, 40). Teuten et al. (41) also concluded that various fish species consume varying amounts of MPs due to differences in nutrition and consumption patterns. According to the findings of a study conducted in Bangladesh, the prevalence of MP was significantly higher in demersal fishes, which are fish that live and feed on or near the bottom of the water in an aquatic environment, in comparison to benthopelagic and pelagic fishes. Demersal fishes were also found to have significantly higher levels of MP than benthopelagic fishes (8). Due to the fact that the sediment beneath the water serves as a sink for MPs and thus contains a significant amount of MPs, demersal and benthoplagic fish may have been subjected to more MPs than pelagic species (20).

In addition, the feeding habits of the fish also influence the total of MP's. The consumption of microplastics by fish may be influenced by feeding patterns, the habitat of fish, and the abundance of plastic material in the aquatic system (17). Furthermore, the habit of omnivores, carnivores, and herbivores may be responsible for a higher percentage of MP consumption (20). *O. niloticus, L. hoeveni,* and *A. testudineus* were omnivores while *L. hoeveni* was carnivore. Consequently, in this study, omnivore was the highest among the MPs. Previous research has shown that omnivorous fish collect a broader range of MPs than herbivorous and carnivorous fish (42). In overall, when considering diet, omnivorous fish had the greatest MPs content, while carnivorous fish had the lowest MPs content.

The findings of this research indicated that the feeding zone (demersal, benthopelagic, and pelagic) and feeding habit (carnivore, omnivore, and herbivore) play significant roles in the consumption of MP by a variety of fish species. This finding indicates the abundance of high-density MPs and other polymers at the pond's bottom. This finding is consistent with previous research on fish freshwater from China (20) and the fish freshwater from Thames River in London (43).

Based on the results from FTIR, polypropylene (PP) was the common polymer found in freshwater. These results can be compared to previous studies that analyzed MPs in freshwater habitats, which found 35.7% of total MPs in the Pearl River (2), 30.6% in the Manas River, 39% in the Qin River, 29.4% in the Antu River, and 4% in the Saigon River (20). Additionally, previous studies stated that 24% of the significant components of MPs found in freshwaters globally were considered PP material (12) which mainly produces packaging materials and fishing equipment (3).

Polyethylene (PE) also was dominant in this study, and Plastics Europe stated that PE is one of the most produced plastics in the world. Many potential sources of PE in the water could be traced back to human fishing activities, such as the use of fishing tools and other plastic product in the vicinity of the water. For instance, researchers discovered a significant amount of PET, PP, and PS in Dongshan Bay, an area that is extensively utilized for aquaculture (39).

Conclusion

In this study, MPs were present in different fish species: *Siluriformes sp., Oreochromis niloticus, Leptobarbus hoevenei,* and *Anabas testudineus.* The MPs abundance was determined to be 29.2% in the GIT of 12 different fishes. Film was the highest morphotype at 35% of the total MPs. White was the dominant color at 28.2% MPs, and MPs size < 500 ***µm*** was the highest at 57.5%. MPs abundance was influenced by the different feeding zones of fish: the abundance in the demersal fish *A. testudineus* was the highest followed by pelagic fishes *Siluriformes sp* and *L. hoeveni;* the lowest was benthopelagic which is *O. niloticus*. The MPs detected in GIT of the collected fishes are largely PE and PP, with sources of urban waste and consumer usage.

This work significantly adds to our understanding of the possible effects of plastic pollution in freshwater at a fishpond near the edge of a paddy field and the factors influencing MPs ingestion by fish.

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Competing interests

The authors declare that they have no competing interests.

Ethical clearance

We obtained ethical approval from the chair of research ethics, Faculty of Health Sciences, UiTM. The ethics number is FERC/FSK/EM/2022/0029.

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References

1. Garcés-Ordóñez O, Saldarriaga-Vélez JF, Espinosa-Díaz LF, Patiño AD, Cusba J, Canals M, *et al*. Microplastic pollution in water, sediments and commercial fish species from Ciénaga Grande de Santa Marta lagoon complex, Colombian Caribbean. Science of the Total Environment. 2002; 829(2):154643.
2. Lam TWL, Fok L, Ma ATH, Li HX, Xu XR, Cheung LTO, & Wong MH. Microplastic contamination in marine-cultured fish from the Pearl River Estuary, South China. Science of the Total Environment. 2002; 827:154281.

3. Lv W, Zhou W, Lu S, Huang W, Yuan Q, Tian M, *et al*. Microplastic pollution in rice-fish co-culture system: A report of three farmland stations in Shanghai, China. Science of the Total Environment. 2019; 652:1209–1218.
4. Nam Phuong N, Thuy Duong T, Phuong Quynh Le T, Kien Hoang T, My Ngo H, Anh Phuong N, *et al*. Microplastics in Asian freshwater ecosystems: Current knowledge and perspectives. 2022 Feb 20; 808:151989
5. Zainuddin AH, Aris AZ, Rozaimi M, Zaki M, Yusoff FM, & Wee Y. Occurrence, potential sources and ecological risk estimation of microplastic towards coastal and estuarine zones in Malaysia. 2022 Jan 1; 174:113282.
6. Mitrano DM & Wohlleben W. Microplastic regulation should be more precise to incentivize both innovation and environmental safety. Nature Communications. 2020; 11(1):1–12.
7. Smith M, Love DC, Rochman CM, & Neff RA. Microplastics in seafood and the implications for human health. Current Environmental Health Reports. 2018; 5(3):375.
8. Ibrahim YS, Rathnam R, Anuar ST, Mohd W, Wan A, & Khalik M. Isolation and characterisation of microplastic abundance in lates calcarifer from Setiu Wetlands, Malaysia. Malaysian Journal of Analytical Sciences. 2017; 1(5):1054–1064.
9. Free CM, Jensen OP, Mason SA, Eriksen M, & Williamson NJ. High-levels of microplastic pollution in a large, remote, mountain lake. Marine Pollution Bulletin. 2014; 85(1):156–163.
10. Parvin F, Jannat S, & Tareq SM. Abundance, characteristics and variation of microplastics in different freshwater fish species from Bangladesh. Science of the Total Environment. 2021 Aug 25; 784:147137.
11. Li J, Ouyang Z, Liu P, Zhao X, Wu R, Zhang C, *et al*. Distribution and characteristics of microplastics in the basin of Chishui River in Renhuai, China. Science of The Total Environment. 2021; 773:145591.
12. Jones TT, Work TM, Brignac KC, Royer SJ, Hyrenbach KD, Jensen BA, *et al*. Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. Marine Pollution Bulletin. 2018; 127:704–716.
13. Yang Hwi T, Shuaib Ibrahim Y, & Wan Mohd Khalik WMA. Microplastic abundance, distribution, and composition in Sungai Dungun, Terengganu, Malaysia. Sains Malaysiana. 2020; 49(7):1479–1490.
14. Park T-J, Kim M-K, Lee S-H, Lee Y-S, Kim M-J, Song H-Y, *et al*. Occurrence and characteristics of microplastics in fish of the Han River, South Korea: Factors affecting microplastic abundance in fish. Environmental Research. 2022; 206:112647.
15. Schmid K, Winemiller KO, Chelazzi D, Cincinelli A, Dei L, Giarrizzo T. First evidence of microplastic ingestion by fishes from the Amazon River estuary. Marine Pollution Bulletin. 2018 Aug 1; 133:814-21.
16. Phillips, Melissa B., and Timothy H. Bonner. Occurrence and amount of microplastic ingested by fishes in watersheds of the Gulf of Mexico. Marine pollution bulletin. 2015; 1:264-269.
17. Szymańska M, Obolewski K. Microplastics as contaminants in freshwater environ- ments: A multidisciplinary review. Ecohydrol Hydrobiol. 2020 Jul 1; 20(3):333-45.
18. Kasamesiri P & Thaimuangpho W. Microplastics ingestion by freshwater fish in the Chi River, Thailand. International Journal of GEOMATE. 2020; 18(67):114–119.
19. Sulistyowati L, Nurhasanah Riani E, & Cordova MR. The occurrence and abundance of microplastics in surface water of the midstream and downstream of the Cisadane River, Indonesia. Chemosphere. 2022; 291:133071.
20. Wang S, Zhang C, Pan Z, Sun D, Zhou A, Xie S, *et al*. Microplastics in wild freshwater fish of different feeding habits from Beijiang and Pearl River Delta regions, south China. Chemosphere. 2020; 258:127345.
21. Najihah M, Ismail MS, Yap CK, Ku KK. Microplastics Occurrence in Waters off the Northwest Coast of Peninsular Malaysia: A Spatial Difference. Journal of Basic & Applied Sciences. 2020; 16(1):50-60.
22. Usman S, Razis AFA, Shaari K, Amal MNA, Saad MZ, Isa NM, *et al*. Microplastics pollution as an invisible potential threat to food safety and security, policy challenges and the way forward. International Journal of Environmental Research and Public Health. 2020; 17(24):9591.
23. Pérez-Reverón R, González-Sálamo J, Hernández-Sánchez C, González-Pleiter M, Hernández-Borges J, & Díaz-Peña FJ. Recycled wastewater as a potential source of microplastics in irrigated soils from an arid-insular territory (Fuerteventura, Spain). Science of The Total Environment. 2022; 817:152830.
24. Zhao M, Cao Y, Chen T, Li H, Tong Y, Fan W, *et al*. Characteristics and source-pathway of microplastics in freshwater system of China: A review. Chemosphere. 2022; 297:134192.
25. Liu J, Yang Y, An L, Liu Q, & Ding J. The value of China's legislation on plastic pollution prevention in 2020. Bulletin of Environmental Contamination and Toxicology. 2022; 108(4):601–608.
26. Perteghella A, Gilioli G, Tudor T, & Vaccari M. Utilizing an integrated assessment scheme for sustainable waste management in low and middle-income countries: Case studies from Bosnia-Herzegovina and Mozambique. Waste Management. 2020; 113:176–185.
27. Arisekar U, Shakila RJ, Shalini R, & Jeyasekaran G. Human health risk assessment of heavy metals in aquatic sediments and freshwater fish caught from Thamirabarani River, the Western Ghats of South Tamil Nadu. 2020 Oct 1; 159:111496.
28. Galgani F, Hanke G, & Maes T. Global distribution, composition and abundance of marine litter.

In: Marine Anthropogenic Litter. Springer Cham Heidelberg New York. 2015; 29–56.
29. Lehtiniemi M, Hartikainen S, Näkki P, Engström-Öst J, Koistinen A, & Setälä O. Size matters more than shape: Ingestion of primary and secondary microplastics by small predators. Food Webs. 2018; 17:e00097.
30. Collard F, Gasperi J, Gabrielsen GW, & Tassin B. Plastic particle ingestion by wild freshwater fish: A critical review. Environmental Science and Technology. 2019 Oct 30; 53(22):12974-88.
31. Lusher AL, McHugh M, & Thompson RC. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Marine Pollution Bulletin. 2013; 67(1–2):94–99.
32. Silva-Cavalcanti JS, Silva JDB, França EJ de, Araújo MCB de, & Gusmão F. Microplastics ingestion by a common tropical freshwater fishing resource. Environmental Pollution. 2017; 221:218–226.
33. Nava V & Leoni B. A critical review of interactions between microplastics, microalgae and aquatic ecosystem function. Water Research. 2021; 188:116476.
34. Dawson AL, Kawaguchi S, King CK, Townsend KA, King R, Huston WM, *et al*. Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill. Nature Communications. 2018; 9(1):1–8.
35. Ahrendt C, Perez-Venegas DJ, Urbina M, Gonzalez C, Echeveste P, Aldana M, *et al*. Microplastic ingestion cause intestinal lesions in the intertidal fish Girella laevifrons. Marine Pollution Bulletin. 2020; 151:110795.
36. Huang W, Song B, Liang J, Niu Q, Zeng G, Shen M, *et al*. Microplastics and associated contaminants in the aquatic environment: A review on their ecotoxicological effects, trophic transfer, and potential impacts to human health. Journal of Hazardous Materials. 2021; 405:124187.
37. Wu C, Xiong X, Hamidian AH, Zhang Y, & Xu X. A review on source, occurrence, and impacts of microplastics in freshwater aquaculture systems in China. Water Biology and Security. 2022; 1(3):100040.
38. Neves D, Sobral P, Ferreira JL, & Pereira T. Ingestion of microplastics by commercial fish off the Portuguese coast. Marine Pollution Bulletin. 2015; 101(1):119–126
39. Pan Z, Liu Q, Jiang R, Li W, Sun X, Lin H, Jiang S, Huang H. Microplastic pollution and ecological risk assessment in an estuarine environment: The Dongshan Bay of China. Chemosphere. 2021 Jan 1; 262:127876.
40. Rummel CD, Löder MGJ, Fricke NF, Lang T, Griebeler EM, Janke M, et al. Plastic ingestion by pelagic and demersal fish from the North Sea and Baltic Sea. Marine Pollution Bulletin. 2016; 102(1):134–141.
41. Teuten EL, Saquing JM, Knappe DRU, Barlaz MA, Jonsson S, Björn A, *et al*. Transport and release of chemicals from plastics to the environment and to wildlife. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 2009; 364(1526):2027–2045.
42. Bellas J, Martínez-Armental J, Martínez-Cámara A, Besada V, & Martínez-Gómez C. Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. Marine Pollution Bulletin. 2016; 109(1):55–60.
43. Wardlaw CM, Corcoran PL, Neff BD. Factors influencing the variation of microplastic uptake in demersal fishes from the upper Thames River Ontario. Environmental Pollution. 2022 Nov 15; 313:120095.