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Microplastic Distribution and Characteristics in Common Carp (*Cyprinus carpio*) from Han River, South Korea

Jung-Keun Oh , Jangho Lee, Soo Yong Lee , Tae Kyung Kim, David Chung and Jinwon Seo *

Natural Environment Research Division, National Institute of Environmental Research, Hwangyeong-ro 42, Seo-gu, Incheon 404-708, Republic of Korea; rightroot@korea.kr (J.-K.O.); ficedula01@korea.kr (J.L.); randol84@korea.kr (S.Y.L.); taek0501@korea.kr (T.K.K.); david426@korea.kr (D.C.)

* Correspondence: jinwonseo91@korea.kr; Tel.: +82-10-9291-3828

Abstract: This study assessed the distribution of microplastics (MPs) in the gills, intestines, and muscles of the common carp (*Cyprinus carpio*), one of main fish species consumed by humans living in the lower regions of the Han River in South Korea. In total, 891 MP particles were detected in 15 carps, with an average of 59.4 ± 45.5 particles/specimen, indicating severe MP contamination. The predominant MP form was fragment (86%), and the size range was 0.02–0.10 mm. Thirty MP particles were detected in the muscle samples (n = 10), primarily in sizes < 0.1 mm (89%). The most common types of polymers detected in this study were polyethylene (≥42%), polystyrene (≥20%), and polypropylene (≥19%), which were at the same level as the polymer ratio of domestic plastic production/use. No correlation was observed between the length of common carp and the number of MPs detected in different body tissues, indicating an abundance of MPs in the environment rather than by residue and accumulation. These findings contribute to the evaluation of the potential impact of edible fish on human health and emphasize the need to develop strategies to reduce MP contamination originating from various potential land sources.

Keywords: MPs; common carp; gill; intestine; muscle; waste plastic recycling

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1. Introduction

Global plastic production has reached a total of 390.7 Mt over approximately 70 years since the 1950s, and studies have estimated that plastic waste produced from 2019 to 2060 will amount to 353.3 Mt [1]. However, only approximately 21% of collected plastic waste is recycled or incinerated, whereas 79% accumulates in landfills or the environment [2,3]. By 2030, the annual discharge of plastics into the aquatic environment of 173 countries is estimated to reach 20–52 Mt [4]. Plastic waste is transported by floods and wind into rivers and oceans, contaminating aquatic ecosystems and threatening aquatic life and biodiversity, especially in rivers near large cities with a high concentration of plastic debris [5,6]. Plastics exposed to aquatic environments undergo physical degradation and break down into MPs (<5 mm in diameter of plastic); this degradation is largely driven by physicochemical reactions such as photo- and biodegradation [7,8].

MPs are ubiquitous in the environment because of their diverse emission pathways. Therefore, their mitigation is a pressing issue worldwide [9]. MPs have been detected in the environment, organs, and tissues of aquatic organisms and humans [10–13]. The contamination routes of MPs in the aquatic environment include the gills and digestive systems of aquatic organisms exposed to MPs, predation on low trophic level organisms exposed to MPs, and direct adhering of MPs to organisms [14]. Trophic level and habitat are known biological factors that influence MP uptake by organisms [15]. Of particular importance, MPs exist in all levels of aquatic ecosystems and pose threats to key species [16].

MPs have been found in edible fish and can exist at high levels in the human body owing to the effects of biomagnification [17,18]. Exposure of fish to MPs can induce changes in immunity, growth, feeding activity, swimming, and reproduction [19,20]. In particular, the neurotoxic effects of MP exposure in fish have been confirmed based on the acetylcholinesterase (AChE) activity measured under laboratory conditions [21,22].

MPs can also impact the predatory behavior of fish because the plastic particles can be confused with real prey, leading to malnutrition and MP accumulation in major organs such as gills, intestines, and stomachs [23–25]. Additionally, plastic nanoparticles ingested by fish through the aquatic food chain can have serious effects on their feeding and schooling behavior as well as their metabolism [26]. The presence of MPs in commercially important fish poses a potential risk to human health owing to the accumulation of MPs and associated pollutants [27]. As fish are a primary source of protein for humans, the presence of MPs in fish and their ecotoxicological effects could impact aquatic food security [28,29].

It has been widely reported that MPs have the ability to combine with pollutants that are considered potentially hazardous in the aquatic environment [30]. In addition to the direct impact of MP itself, the negative effects of MP are also determined by additives added during the manufacture of plastic products or contaminants adsorbed on plastic debris exposed to the environment [31]. MPs also carry organic pollutants and trace metals into aquatic habitats [32]. Because of their high adsorption capacity, plastic particles can adsorb hydrophobic persistent organic pollutants (POPs) on their surface at concentrations higher than other pollutants in water [33,34]. In aquatic environments, MPs can absorb and concentrate toxic organic substances, increasing their toxicity tenfold and posing a severe risk to human health [35]. Therefore, MP distribution in edible fish should be monitored to identify contamination characteristics and exposure pathways of MPs to humans.

The common carp (*Cyprinus carpio*) is an important source of protein for humans and is consumed globally through various cooking methods, such as frying and steaming. In South Korea, common carp are generally prepared in a cauldron containing oil to remove the fishy odor; the whole fish is boiled for more than 10 h, and its broth is consumed for postnatal care. The aim of this study was to investigate the distribution of MPs in common carp and to identify the characteristics of MP pollution in the targeted regions based on MPs detected in this fish species.

2. Materials and Methods

2.1. Sampling Area and Collection

The Han River, which flows through Seoul, is one of South Korea's four major rivers. The Han River basin occupies approximately 27% (26,219 km^2) of the country's area and provides drinking water to over 50% of the country's population (over 26 million people) [36]. Approximately 34% of the total nationwide recycling companies related to plastic waste and waste electrical and electronic products are distributed near the middle and lower regions of the Han River [37], and the two facilities with the highest wastewater discharge in South Korea are located in the lower regions of the river [38].

To investigate the distribution of MPs in common carp, we collected 15 male specimens using gillnets in the lower regions of the Han River in South Korea between July and August 2020 (Figure 1). The collected fish were brought to the laboratory, where their weight and length were measured. The muscle, gills, and intestines of the fish were immediately dissected and stored in stainless steel cans until preprocessing. To minimize plastic background contamination, all tools used were made of stainless steel and cleaned with deionized water and organic solvents before use.

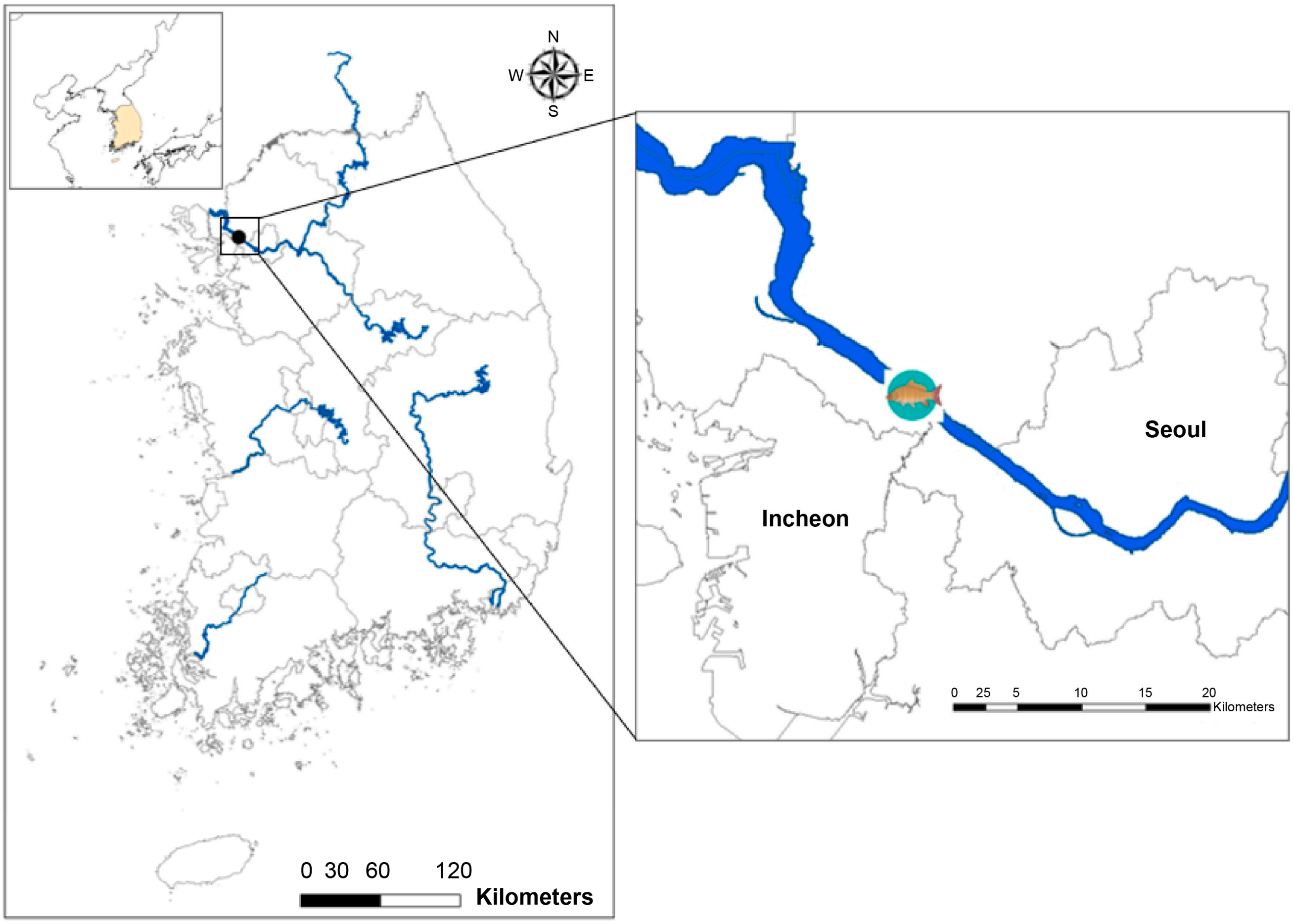

Figure 1. Location of the sampling site in the Han River.

2.2. Reagents and Experiments

All preparation and experiments were conducted in high-efficiency particulate absorbing (HEPA)-filter positive-pressure facilities with electrostatic discharge (ESD)-resistant flooring to minimize external and cross-contamination from the air and researchers. The distilled water and 10% KOH solution used to dissolve the samples were filtered once through a GF/F filter (pore size = 0.7 µm) and stored in a clean beaker covered with foil. Lithium metatungstate (LMT) solution was used for MP density separation. The LMT solution was adjusted to a 1.6 g/cm^3 density by mixing 1 L of LMT solution with a 2.95 g/cm^3 density with 2.25 L of distilled water. The glassware used in the experiments was surface-cleaned with HPLC-grade water and covered with foil for storage until use. The sieve used for MP separation was initially cleaned with ethanol and an ultrasonic cleaner, followed by secondary cleaning with an air gun, and then wrapped in aluminum foil. In this study, the entire amount of the gill and intestine dissected from an individual specimen was used as the sample. The muscle samples were first weighed and then cut into less than 1 cm pieces using a scalpel. Samples were placed in a beaker and submerged in a 10% KOH solution to break down proteins. The beaker was then placed on a hotplate maintained at 75 °C until the solid sample fully dissolved into a liquid state inside a fume hood. After the reaction was complete and the solution cooled to room temperature, the solution was filtered through a 20 µm sieve and then rinsed at least three times with distilled water to remove any remaining KOH solution. After protein decomposition, the sample filtered through the 20 µm sieve was transferred onto a density separation funnel using the LMT solution. All particles on the sieve were transferred to the funnel through repeated rinsing (at least three times). More than 150 mL of solution was separated to enable upper- and lower-layer separation in the funnel. The funnel opening was wrapped with aluminum foil and allowed to settle for 24 h. The lower layer was removed to a minimum volume if the upper and lower layers were separated. After installing a stainless- steel mesh filter (20 µm Φ) in the filtration device, samples that had undergone density separation were gradually poured in for filtration. After filtration, the filter was placed in a glass or aluminum cup, wrapped in foil, and then dried in an oven at 40 °C for 12 h. The dried samples were stored in a desiccator until further analysis.

2.3. Analysis

The analytical equipment used to analyze the samples was a Thermo Fisher Scientific Nicolet iN10 infrared microscope (software: Omnic Picta version 1.7.). The analysis followed the KS M 0024: 2017, a Korean standard for infrared spectrophotometric analysis [39]. The entire surface of the filter sample was scanned using Fourier-transform infrared spectroscopy (FT-IR) ultrafast mapping (in transmission mode). Material identification was confirmed through a profile analysis, followed by a qualitative and quantitative analysis of the sample particles. The total count of MPs in each tissue (gills: n = 15; intestines: n = 15, muscles: n = 10) of the 15 common carps was calculated by subtracting the number of blanks from the MPs in each sample. The blank tests were divided into field blank and procedure blank. The field blank was placed in the process from dissecting the carp to placing the sample in the stainless steel bottle, and the procedure blank was prepared in the pretreatment process after taking it out of the stainless steel bottle, weighing it, and placing it in a glass jar. The MPs detected in each organ were calculated based on the shape, polymer type, and size. The forms of MPs were categorized into fragments and fibers derived from the breakdown of larger plastic products [40]. Other shapes like films, foams or microbeads did not occur. Thirteen different polymers were identified and the size of MPs was divided by 5 categories: <20, 20–200, 200–500, 500–1000, and >1000 µm.

2.4. Quality Assurance & Quality Control (QA/QC)

Only stainless steel and glassware were used to prevent external MP contamination, and latex gloves and cotton lab coats were worn during the experiments. Before use, the distilled water underwent triple distillation and was filtered through a GF/F filter with a pore size of 0.75 µm. For internal quality control in the laboratory, the blank tests for three types of samples (liquid, organic, and air) were performed. In the case of liquid, distilled water was assumed to be the sample and performed the analysis after filtering. In the case of organic matter, the result was analyzed through a decomposition process with KOH. In the case of air, we analyzed the distilled water in a glass petri dish or stainless steel container after leaving it for 24 h while opened in the laboratory space. The detection limit value was maintained below one for seven types of polymers in each blank sample (Table S1). Quality control was conducted using plastic standard samples for polyethylene (PE) and polystyrene (PS), with recovery rates of 100% and 94% ± 1.6%, respectively. The organic material decomposition using KOH can last up to 3 weeks, depending on the sample condition. To identify potential plastic deformation attributed to KOH, 20 PE and 20 PS standard samples (430 µm size) were placed in a beaker with 100 mL of KOH and heated to 75 °C. The volume was maintained at 100 mL until the samples melted. The surface and shape of the standard samples remained unchanged.

3. Results and Discussion

3.1. MP Concentration

We found 891 MP particles in the 15 common carp that were studied. In addition, the total number of MP particles detected in the gills, intestines, and muscles of specimens were 316 (35%), 545 (61%), and 30 (3%), respectively (Table S2). Furthermore, MP particles were detected in the gills, intestines, and muscles at frequencies of 93%, 100%, and 60%, respectively. The average concentration of MPs by weight was the highest in gills (1.60 ± 1.69 particles/g), followed by intestines (1.34 ± 1.64 particles/g) and muscles (0.12 ± 0.13 particles/g) (Table 1).

Table 1. Abundance, shape, and size of MPs in common carp measured in this study.

Tissue	MPs/Fish	MPs/g (SD)	Shape (%)	Most Abundant Polymer * Type (%)	Most Abundant MP Size (μm) (%)
Gill (n = 15)	316	1.60 ± 1.69	Fragment (95) Fiber (5)	PE (85)	Fragment: 20–50 (55) Fiber: 50–100 (41)
Intestine (n = 15)	545	1.34 ± 1.64	Fragment (86) Fiber (14)	PP (29), PS (29)	Fragment: 50–100 (33) Fiber: 600–700 (8)
Muscle (n = 10)	30	0.12 ± 0.13	Fragment (93) Fiber (7)	PEVA (53)	Fragment: 20–50 (50) Fiber: ≥1000 (100)

* PE: Polyethylene, PP: Polypropylene, PS: Polystyrene, PEVA: polyethylene vinyl acetate.

The level of MP contamination (59.4 ± 45.5 particles/specimen) in the common carp of this study was notably higher than those observed at the same sampling location in previous studies (28.5 ± 13.0 particles/specimen) [41,42], indicating severe contamination of common carp by MPs in the lower Han River. Recent studies conducted in the same area [43–45] reported high levels of MPs in the water and sediments of the aquatic environment, suggesting that common carp, which inhabit the riverbed, are likely to ingest MPs from environmental media [46].

The high level of MP contamination in the studied carps could be attributed to the increasing use and disposal of terrestrial plastics. Compared to existing literature conducted in the same water system, the levels of MPs in the studied common carp have shown a yearly increasing trend [43,47]. Plastic consumption in South Korea has increased yearly, especially since the coronavirus disease (COVID-19) outbreak, which led to the generation of single-use plastic waste. Moreover, the material recycling rate for household waste, which mainly consists of single-use plastics, is only approximately 16%, indicating the potential future impact on the atmospheric and water quality due to the increasing presence of plastic particles [48].

Owing to the absence of appropriate environmental standards for MPs, fragments from plastic treatment and recycling processes can enter rivers through rainwater or sewage treatment plants (STPs). Two of Seoul's largest STPs are located in the lower Han River and process 1,630,000 and 860,000 m^3/d of wastewater [49]. Given that domestic STPs are a vital source of MPs [39], large STPs substantially correlate with the MPs detected in the nearby water and fish.

3.2. MP Distribution (Shape, Type, and Size)

The detected MPs were either classified as fragments or fibers according to their shape: 798 fragments (90%) and 93 fibers (10%) (Figure S1) were found. In all sections, fragments accounted for more than 86% of the shapes, consistent with recent studies that reported that the majority of the MPs found in common carp species are fragments [43,47]. Twelve types of plastic materials were detected in the samples, with the most common polymers being PE (≥42%), PS (≥20%), and polypropylene (PP; ≥19%) (Table S3). The plastic materials varied according to specific tissue of the fish in which they were found. For instance, six different materials were found in the gills and muscles, among which PE (85%) and polyethylene vinyl acetate (PEVA; 53%) were the predominant materials. In addition, 11 different types of MP materials were detected in the intestines, with PP and PS (each at 29%) being most frequent (Figure 2). These results are consistent with previous studies conducted at the same location, indicating that the habitat of the common carp has a substantial impact on its MP concentration [50].

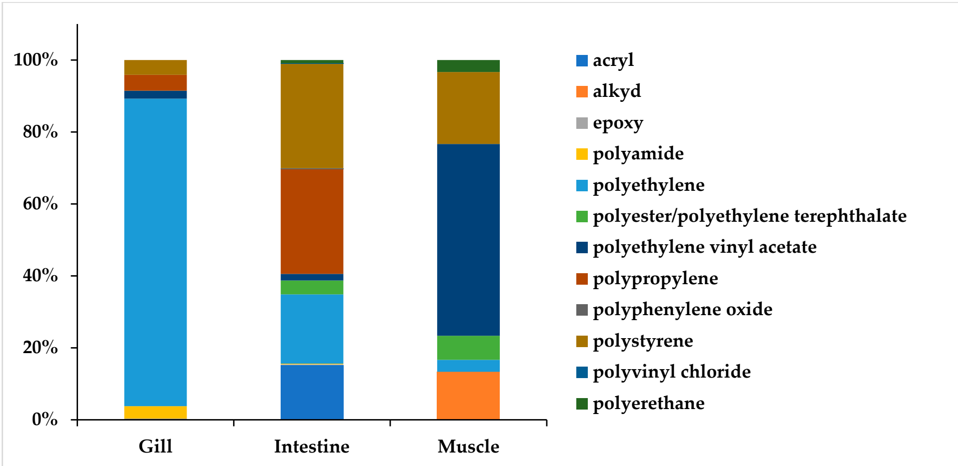

Figure 2. Distribution (%) of polymer type in different tissues of the common carp in the Han River.

When examining the detection frequency based on MP size (Table S4), fragments of 20–50 μm (55%) and fibers of 50–100 μm (41%) were the most common MP particles found in gills. In the 20–200 μm range, both shapes showed a high detection rate of 99% (fragments) and 82% (fibers). Fragments of 50–100 μm (33%) and fibers of 600–700 μm (8%) were the most detected in the intestines. Fiber MPs in fish gills may be related to the anatomy of the gills, whose comb like structures favor the external adherence of fibers [51]. Fragments were primarily detected in the 20–200 μm range (79%), whereas fibers were primarily detected in the 500–1000 μm range (30%). In muscles, the most common size detected (50%) was 20–50 μm, representing 75% of the MPs detected in the 20–200 μm range. In addition, fragments < 20 μm were most detected in the muscles (21%) compared to the other tissues of the fish. For fibers, only particles 1000 μm or larger were detected, and they were only detected twice (Figure 3).

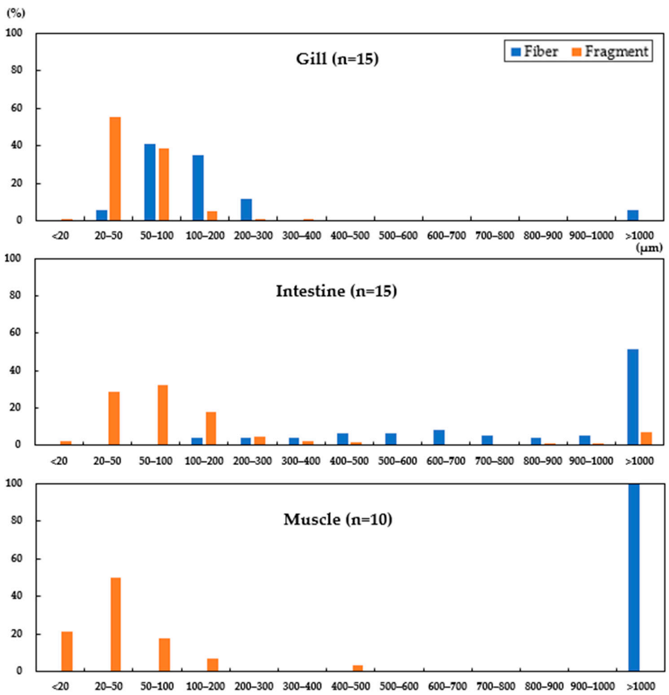

Figure 3. Distribution (%) of polymer size (μm) found in different tissues of the common carp in the Han River.

Previous studies have detected MPs < 100 µm in fish muscles [52–54]. One of the pathways for the entry of MPs into the muscles is through enterocyte absorption, particularly for particles smaller than 10 µm [55,56]. However, the mechanisms underlying the presence and pathways of MPs in muscles remain unclear. Despite the current theories of translocation or adherence [57], further research is required, mainly due to MPs in fish muscles consumed by humans [58]. This study had a limitation in that the number of samples was insufficient to determine statistical significance. Therefore, further research is needed to select representative fish muscles for analysis and to conduct continuous annual monitoring through unified preprocessing and analytical methods to elucidate the distribution characteristics of MPs in fish muscle.

3.3. MP Distribution and Potential Risk

In this study, only non-spherical forms (fragments and fibers) of MPs were detected, which may be more toxic than other forms [59]. Bioaccumulation is a critical factor in the potential ecological risks of MPs. According to recent studies, there is no correlation between the size or weight of fish and their concentration of MPs [58,60]. In contrast, other studies have reported that the MP concentration in fish increases with their weight [61,62]. We measured MP concentrations across different fish sizes and tissues to identify potential bioaccumulation based on size and found no significant correlation (Figure 4). It is challenging to relate recent studies indicating that omnivorous common carp contain more MPs than carnivorous fish to bioaccumulating MPs along the food web in freshwater ecosystems [43,63]. In the case of carp, they live at the bottom of rivers and filter out sediment during feeding activity. Sediments may have higher levels of contamination of MPs than water or living organisms, indicating that omnivore carp show higher contamination levels than carnivorous fish species. The lack of a strong correlation between fish size and the MPs found in their intestines might occur because ingested MPs are likely rapidly expelled from organs such as the intestines, resulting in fewer long-term residues [64]. Moreover, the concentration of MPs within the intestines varies greatly between specimens, further impeding the identification of potential correlations between fish size and MP concentration [62].

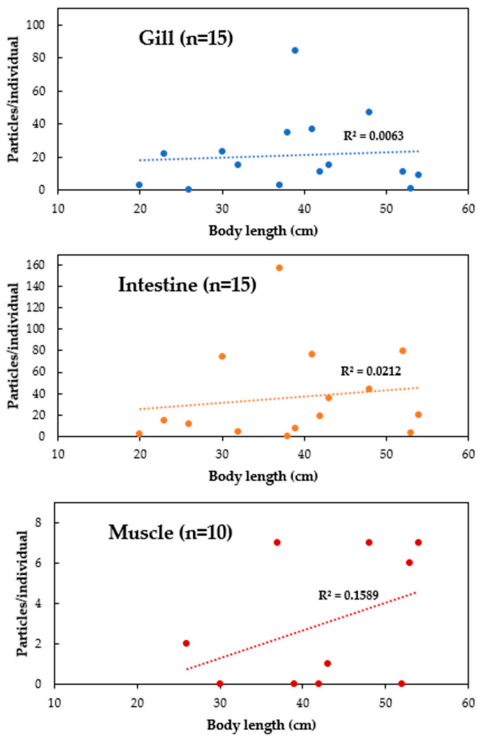

Figure 4. Correlation between number of MP particles and body length of common carps.

Several studies have reported that MPs can adsorb heavy metals and organic compounds and concentrate these pollutants at higher levels than the surrounding environment. Therefore, MPs are potential vectors for contamination of aquatic life [33,65]. Given that common carp can reflect the level of the MP and chemical pollution in the environment at the time, the absence of standards and treatments for reducing MPs in STPs implies that humans cannot avoid MP pollution unless they refrain from consuming fish.

3.4. Need for Continuous Monitoring of MPs in Fish

To confirm temporal changes in MPs in carp, the results were compared with previous studies conducted in the same area. [43]. The distribution of MPs (59.4 $\pm$ 45.5 particles/specimen) in carp collected in 2020 (this study) was more than twice as high as the distribution of MPs (28.5 $\pm$ 13.0 particles/specimen) in carp collected in 2019 (previous study). Upon material-specific examination, the ratios of PE and PP remained >60%, but PS and PEVA, which previously had low detection rates, emerged as dominant materials in the intestines and muscles of fish, respectively.

PE and PP are produced and used in plastic bags and packaging films, whereas PS is commonly used in food packaging and electrical appliances, and PEVA is an alternative to PVC. In South Korea, the COVID-19 outbreak dramatically increased the packaged and delivered food culture. As a result, plastic waste increased by approximately 50%, and the use of plastic bags increased by 16% in 2020 compared to 2017, amounting to 27.6 billion units (0.55 million tons) [48]. The per capita production of daily plastic waste more than doubled from 110 g in 2016 to 236 g in 2020 [66]. Although there are constraints in directly comparing previous research with the findings of the present study, the results indicate that continuous monitoring through ecological samples is crucial to clarify the environmental impacts of plastics.

According to a Greenpeace report [48], if this increasing trend continues, household plastic waste is expected to reach approximately 6,475,000 tons by 2030, representing a 1.47-fold increase compared to that in 2020. Owing to the presence of four major STPs along the Han River in Seoul, the possibility of elevated MP distribution in freshwater and fish in the future is high. Furthermore, recycling companies for plastics and electronic products are concentrated in the Seoul Capital Area, where tributaries of the Han River are located. Therefore, microdust generated in the recycling process and plastic fragments that fall on the ground can enter the waterways during rainfall. Because these particles are more fragmented than waste plastic products, they are more prone to further degradation in aquatic environments, which negatively impacts the environment and ecosystems. Therefore, appropriate regulations should be implemented to prevent the release of MPs into the environment during recycling and treatment processes. Moreover, measures should be implemented to reduce plastic production and usage to mitigate environmental pollution caused by MPs.

Despite this being a detailed and comprehensive study, it does have some limitations. For example, despite thorough laboratory quality control, it cannot be ruled out that there is a possibility of external contamination of MPs. In the case of fish gills in particular, the possibility of contamination still remains because information is unknown as to whether it is attached from the outside or incorporated within the tissue. Therefore, selection of representative samples is important, and careful attention to prevent external contamination is required during the overall experiment process. Also, the characteristics of the pollution source could not be identified owing to the lack of data on the amount of macro- and MPs generated from various potential pollution sources (e.g., STPs, plastic production, recycling facilities). Further research is warranted to estimate the amount of macro- and MPs released into the environment in land-based (waste) plastic and recycling/processing facilities in order to formulate effective strategies to mitigate MP pollution in aquatic environments.

4. Conclusions

This study investigated the distribution of MP pollution in common carp inhabiting the lower regions of the Han River in South Korea. The results provide a comprehensive understanding of MP contamination in different tissues of the common carp. Increased levels of MPs were detected in the gills, intestines, and muscles of the examined common carp, exceeding the levels reported in previous studies focusing on this particular species in the same area. The elevated levels of MP contamination in common carp can be attributed to the remarkable increase in plastic use and disposal following the COVID-19 outbreak, discharge from domestic STPs, and potential environmental exposure during plastic recycling. Considering the weak correlation between the types of MPs detected and the concentrations across different carp sizes, it is difficult for common carp to serve as a bio-indicator reflecting the level of microplastic MP contamination in aquatic ecosystems. However, given that carp are harvested for food and the entire carp is consumed by humans, continuous monitoring of this fish species is necessary from the perspective of human health effect. Both fragments and fibers of MPs were detected in the fish muscles in this study. Considering the widespread consumption of common carp by humans, continuous retrospective monitoring is essential to assess the risks associated with MP pollution in aquatic environments to human health.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15234113/s1, Figure S1. Shapes of MPs in fish samples from Han River, South Korea; Table S1. Detection limit values for each type of samples (liquid, organic matter, and air) in the blank test; Table S2. Microplastic concentrations by different tissues [gill (n = 15), intestine (n = 15), and muscle (n = 10)] of common carps in the Han River, South Korea; Table S3. Polymer compositions by different tissues (gill, intestine, and muscle) of common carps in the Han River, South Korea; Table S4. Microplastic distributions of shape and size (μm) by different three tissues (gill, intestine, muscle) of common carps in the Han River, South Korea.

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