

From Pond to Plate: Laser Direct Infrared (LDIR) Chemical Imaging System Characterization and Quantification of Microplastics in Farmed Common Carp (*Cyprinus carpio* Linnaeus, 1758)

DEISLAVA ARNAUDOVA¹, STELA STOYANOVA³, ELENKA GEORGIEVA³, VESELA YANCHEVA^{2*}

¹Paisii Hilendarski University of Plovdiv, Faculty of Biology, Department of Zoology, 24 Tsar Assen Str., 4000 Plovdiv, Bulgaria,

^{2*}Paisii Hilendarski University of Plovdiv, Faculty of Biology, Department of Ecology and Environmental Conservation, 24 Tsar Assen Str., 4000 Plovdiv, Bulgaria, vyancheva@uni-plovdiv.bg

³Paisii Hilendarski University of Plovdiv, Faculty of Biology, Department of Developmental Biology, 24 Tsar Assen Str., 400, Plovdiv, Bulgaria

Abstract. Microplastic (MP) contamination has become a critical environmental issue with increasing relevance to food safety and the sustainability of aquaculture. This study aimed to assess for the first time in Bulgaria the occurrence, distribution, and polymer composition of MPs in muscle tissue of Common carp (*Cyprinus carpio* Linnaeus, 1758), cultivated in aquaculture systems. The edible tissues were analyzed using Laser Direct Infrared (LDIR) imaging spectroscopy, an advanced technique enabling rapid and accurate particle identification. MPs were detected in the muscle of all examined specimens, confirming their bioavailability and potential transfer along the food chain.

Keywords: microplastics, Common carp, aquaculture.

Introduction

The Common carp, a notable freshwater species, has been cultivated for over 8000 years and is among the most extensively farmed fish globally (Nakajima *et al.* 2019), including in Bulgaria. Favoured by consumers for its adaptability, rapid growth, and high protein content, this fish is integral to global fisheries and aquaculture. It is the third most important aquaculture species in the world (FAO, 2020), and as one of the dominant Cyprinid species, the Common carp is cultured in over 100 countries, with a total production of 3 million metric tons (Bostock *et al.* 2010). The Common carp, which is widely distributed worldwide and has a significant economic impact and market share, has also been designated as an international test organism for aquatic toxicology studies (OECD, 1993). The widespread use of plastic products worldwide, coupled with improper waste recycling management and inadequate policy regulations, has led to widespread pollution by microplastics (MPs: less than 5 mm) in the environment, posing a major environmental challenge in the 21st century and significant threats to global ecosystems (Wang *et al.* 2020; Chiani *et al.* 2025). In aquaculture environments, MPs originate from multiple sources including terrestrial plastic waste, byproducts of fishing activities, plastic products used in aquaculture operations, and atmospheric deposition (Chen & Wang 2021). Plastic items like fishing nets, buoys, and feed bags used in aquaculture can wear and age during use, releasing MP particles into the culture water (Peng *et al.* 2024).

This pilot study aimed to assess for the first time in Bulgaria the occurrence and polymer composition of MPs in the muscle tissue of the farmed freshwater Common carp.

Materials and methods

Five adult Common carps (mean weight 3150 ± 15.5 g; mean length 45.5 ± 2.5 cm) were obtained from a store for fresh fish in Plovdiv, Bulgaria, where all seafood is delivered daily from various aquaculture farms across the country. Muscle tissue samples were dissected from each specimen and subjected to preliminary treatment for the oxidative degradation of organic matter. Digestion was performed using a 10% potassium hydroxide (KOH) solution combined with 15% hydrogen peroxide (H_2O_2) until complete breakdown of organic material was achieved. Following digestion, the MPs were separated by density using centrifugation in saturated solutions of sodium chloride (NaCl) or zinc chloride ($ZnCl_2$), depending on the expected polymer type. The supernatant layer containing polymeric particles was carefully collected and filtered through a metal-coated membrane filter (aluminum or gold) with a pore size of $0.8 \mu m$. The filtered samples were analysed using a Laser Direct Infrared (LDIR) imaging spectrometer operating in the mid-infrared region. Each filter was initially imaged under visible light to localize the MP particles. Infrared scanning was then performed at a fixed wavelength of 1442 nm to identify residual organic matter and facilitate automated detection of potential MP particles. Subsequently, point-based spectral measurements were conducted for each detected particle. A quantum cascade laser (QCL) sequentially generated infrared radiation in the $975\text{--}1800\text{ cm}^{-1}$ range, covering characteristic absorption bands of most polymers. The spectra were compared against a reference spectral library for reliable polymer identification. Quantitative results were reported as the number of identified particles per sample and as estimated mass, calculated from measured particle area and the density of the corresponding polymer type. All analyses were performed in triplicate. To minimize potential contamination during sampling, processing, and analysis, strict procedural controls were implemented. All equipment and reagents were pre-filtered, procedural blanks were included, and samples were handled under a laminar flow hood using natural fiber lab coats and nitrile gloves. Airborne contamination was monitored to ensure reliable MP identification and quantification. The results are presented as average \pm standard deviation using the program GraphPad Prism 7 for Windows (USA). The results were also analyzed for the significance of differences among the different polymer types by the Kruskal-Wallis test, followed by the Mann-Whitney test (median comparison). The levels of statistical significance were set at $p < 0.05$.

Results and Discussion

Table 1 shows the type and average concentration of each MP polymer, which were detected via LDIR in the Common carp muscle. In total, ten different polymers were detected: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyamide (PA), polyethylene terephthalate (PET), polyvinyl chloride (PVC), acrylonitrile butadiene styrene (ABS), polyurethane (PU), rubber, and polyoxymethylene (POM). In all five samples, polyethylene (PE), polypropylene (PP), polystyrene (PS), polyamide (PA), acrylonitrile butadiene styrene (ABS), and rubber were found, being the dominant polymer type. Polyethylene terephthalate (PET), polyvinyl chloride (PVC), and polyurethane (PU) were found only in two of the analysed samples. In addition, polyoxymethylene (POM) was also found only in two fish muscles. The highest abundance was for polyethylene (PE) – up to 30 pieces/g, polyamide (PA) – up to 40 pieces/g, and rubber – up to 27 pieces/g. The lowest abundance was for polyvinyl chloride (PVC) – 1 piece/g. The other detected polymers had an abundance ranging from 2 to 19 pieces/g.

Table 1. Type and abundance of various MP particles detected in the muscle of farmed Common carp from Bulgaria via Laser Direct Infrared (LDIR) Chemical Imaging System.*Significantly different abundance compared to the other polymer types ($p < 0.05$)

Type	Pieces/g
Polyethylene (PE)	15.33*±11.14
Polypropylene (PP)	6±4.54
Polystyrene (PS)	4.33±3.39
Polyamide (PA)	22.33*±13.27
Polyethylene terephthalate (PET)	7.77±8.33
Polyvinyl chloride (PVC)	0.66±0.47
Acrylonitrile butadiene styrene (ABS)	3.33±0.94
Polyurethane (PU)	2±1.63
Rubber	19.33*±5.55
Polyoxymethylene (POM)	2.66±3.09

The results of our study are the very first for the accumulation of MPs in farmed freshwater fish in Bulgaria. Therefore, we cannot compare it with other results. Toschkova *et al.* (2024) analysed MPs in five commercial fish species from the southern Bulgarian Black Sea. Similarly to our study, the bulk of insulated plastics were made of polyethylene (PE). However, they found a lower abundance of MPs in the fish meat and identified seven MP types, while we identified nine. Yet, some of their samples included, like ours, polyamide (PA, nylon fibres) and polyethylene terephthalate (PET), emphasizing Common environmental problems in both freshwater and marine environments that need to be solved. According to Savoca *et al.* (2019, 2020) and Capillo *et al.* (2020), MPs are more frequently found in the gastrointestinal tract (GIT) of aquatic organisms; however, our findings are concerning because of the high levels of MPs that were detected in the muscle tissue of the sampled fish. Previous studies showed that MPs can accumulate along trophic levels through bioaccumulation and biomagnification (Van Cauwenberghe & Janssen 2014), raising potential concerns for human health through seafood consumption. Savoca *et al.* (2021) isolated (0.11 items/specimen) a total of 9 MPs from the GITs of farmed Common carp - among them, 55.5% from adult individuals. While the presence of MPs in edible fish tissues highlights a possible exposure route, the extent and nature of the associated health risks remain unclear and require further investigation. These results underscore the need for continued monitoring of MP contamination in aquaculture systems and the development of strategies to mitigate potential risks to consumers. However, only a few studies evaluated the MPs and man-made fiber pollution in farmed fish species (Lv *et al.* 2020; Wu *et al.* 2020). Our study, to the best of our knowledge, is the first report on the presence of MPs in farmed Common carp in Bulgaria and complements the results on the two farmed fish, Gilthead sea bream and Common carp, in Croatia by Sovoca *et al.* (2021).

The detection of MPs in the edible portion of farmed Common carp highlights potential implications for human exposure through fish consumption. These findings provide important baseline data on MP contamination in aquaculture fish and underline the need for continuous monitoring and improved management practices to minimize plastic pollution in fish farming environments in Bulgaria.

Acknowledgements: This study is financed by the Department of Scientific Research of University of Plovdiv, project SP25BF001 (СП25-БФ-001) - Pilot study on the quantity and composition of microplastics in freshwater commercially important fish species

(Common carp, *Cyprinus carpio* (Linnaeus, 1758) and brown trout, *Salmo trutta fario* (Linnaeus, 1758) in Bulgaria.

References

- Chen, G., Li, Y. & Wang, J. (2021) Occurrence and ecological impact of microplastics in aquaculture ecosystems. *Chemosphere*, 274: 129989.
- FAO. (2020) State of Fisheries and Aquaculture, State of Fisheries and Aquaculture.
- Capillo, G., Savoca, S., Panarello, G., Mancuso, M., Branca, C., Romano, V., D'Angelo, G., Bottari, T. & Spanò, N. (2020) Quali-quantitative analysis of plastics and synthetic microfibers found in demersal species from Southern Tyrrhenian Sea (Central Mediterranean). *Marine Pollution Bulletin*, 150: 110596.
- Chiani, M.M., Rasta, M., Taleshi, M.S. & Elmi, F. (2025) The role of organisms' size in microplastic pollution monitoring: Insights from *Mytilaster lineatus* and *Amphibalanus improvisus*. *Marine Environmental Research*, 204: 106863.
- Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I. & Corner, R. (2010) Aquaculture: global status and trends. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1554): 2897-2912.
- Lv, W., Yuan, Q., He, D., Lv, W. & Zhou, W. (2020) Microplastic contamination caused by different rearing modes of Asian swamp eel (*Monopterus albus*). *Aquaculture Research*, 51: 5084-5095.
- Nakajima, T., Hudson, M.J., Uchiyama, J., Makibayashi, K. & Zhang, J. (2019) Common carp aquaculture in neolithic China dates back 8,000 years. *Nature, Ecology and Evolution*, 3: 1415-1418.
- OECD (1993) Guideline for the Testing of Chemicals, 203, vol. 1 guideline.
- Peng, X., Li, X., Zhou, J., Tan, J., Chen, G., Zhu, Z. & Yang, T. (2024) Beyond plastic pollution: Unveiling chemical release from plastic debris in river water and seawater using non-target screening. *Water Research*, 267: 122515.
- Savoca, S., Capillo, G., Mancuso, M., Bottari, T., Crupi, R., Branca, C., Romano, V., Faggio, C., D'angelo, G. & Spanò, N. (2019) Microplastics occurrence in the Tyrrhenian waters and in the gastrointestinal tract of two congener species of seabreams. *Environmental Toxicology and Pharmacology*, 67: 35-41.
- Savoca, S., Matanović, K., D'Angelo, G., Vetri, V., Anselmo, S., Bottari, T., Mancuso, M., Kužir, S., Spanò, N., Capillo, G. & Di Paola, D. (2021) Ingestion of plastic and non-plastic microfibers by farmed gilthead sea bream (*Sparus aurata*) and common carp (*Cyprinus carpio*) at different life stages. *Science of the Total Environment*, 782: 146851.
- Toschkova, S., Ibryamova, S., Bachvarova, D.C., Koynova, T., Stanachkova, E., Ivanov, R., Natchev, N. & Ignatova-Ivanova, T. (2024) The assessment of the bioaccumulation of microplastics in key fish species from the Bulgarian aquatory of the Black Sea. *BioRisk*, 22: 17-31.
- Van Cauwenberghe, L. & Janssen, C.R. (2014) Microplastics in bivalves cultured for human consumption. *Environmental Pollution*, 93: 65-70.
- Wang, C., Zhao, J. & Xing, B. (2021) Environmental source, fate, and toxicity of microplastics. *Journal of hazardous materials*, 407: 124357.
- Wu, F., Wang, Y., Leung, J.Y.S., Huang, W., Zeng, J., Tang, Y., Chen, J., Shi, A., Yu, X., Xu, X., Zhang, H. & Cao, L. (2020) Accumulation of microplastics in typical commercial aquatic species: a case study at a productive aquaculture site in China. *Science of the Total Environment*, 708: 135432.