DOI: 10.46793/SBT30.40MN

VISUAL IDENTIFICATION OF MICROPLASTIC CONTAMINATION IN COMMON BLEAK (*Alburnus alburnus*, L. 1758) FROM GRUŽA LAKE

Marijana Nikolić[1], Marija Jakovljević[1], Aleksandra Tubić[2], Branko Kordić[2], Branislav Jović[2], Predrag Simović[1], Vladica Simić[1]

Abstract: Microplastics (MPs) in freshwater ecosystems pose significant environmental risks, particularly to fish populations. This study examines the gastrointestinal (GI) tract contents of fish, revealing a predominance of blue fibers, likely originating from textiles and fishing activities. After visual detection, digestion of residual biological material is needed for precise analysis. Identifying MPs requires chemical techniques like FTIR and Raman spectroscopy. Blood traces in fish intestines suggest potential physiological impacts. As lakes act as pollution sinks, understanding MPs sources is crucial for mitigating risks to aquatic ecosystems and ensuring environmental sustainability.

Keywords: Microplastics, Gastrointestinal tract, Freshwater ecosystems, Morphological classification

Introduction

Microplastic (MPs) pollution in the aquatic environment is a global crisis and is widely present in diverse aquatic habitats worldwide, from the Arctic (Kögel et al., 2023) to the Antarctic (Aves et al., 2022). MPs have been found in all components of ecosystems, including seawater (Taha et al., 2021), marine sediment (Nuamah et al., 2023), beach sand (Aslam et al., 2020), freshwater (Dris et al., 2024), riverine sediment (Maheswaran et al., 2022), lakes (Chen et al., 2024), soil (Ali et al., 2024), and even air (Maurizi et al., 2024). Due to improper management and disposal practices, a large amount of plastic waste enters the environment through various pathways (Geyer et al., 2017). Once it enters the environment, plastic can slowly break down and generate numerous smaller plastic debris under physical, chemical and biological processes (PlasticsEurope 2019). MPs found in natural environments exhibit a high degree of diversity and complexity in terms of type, shape, size, and composition, all of

[1] University of Kragujevac, Faculty of Science, Radoja Domanovića 12, Kragujevac, Serbia
[2] University of Novi Sad, Faculty of Science, Trg Dositeja Obradovića 3, Novi Sad, Serbia

which are believed to influence their toxicity (Lambert et al., 2017). The habitat for plastic accumulation is generally related to plastic waste input, topographical features, and hydrological conditions (Zhang et al., 2021). The accumulation and breakdown of plastics are among our time's most pervasive, persistent, and concerning environmental challenges (Salerno et al., 2021). Since the 1950s, annual plastic production has surged from 1.5 million tons to 450 million tons in 2023 (Ritchie et al., 2023). This rapid increase, combined with the durability of plastics, unsustainable consumption, and inadequate waste management, has led to the extensive buildup of plastic debris in natural environments (David et al., 2009). While the effects on marine environments are well studied, data on freshwater ecosystems, especially their fauna, remain scarce and dispersed (Pinheiro et al., 2017), highlighting the need for further research.

Plastic waste pollution in Serbia's freshwater ecosystems remains largely understudied, despite the widespread use of plastic and the lack of waste management practices aligned with European standards. Under favorable conditions such as oxidation, photo-degradation, mechanical abrasion, and chemical reactions, plastic waste can easily break down from larger items into microplastics (Andrady et al., 2022). MPs contamination in freshwater environments seriously threatens aquatic organisms and overall ecosystem health (Singh et al., 2024). Lingshi et al. (2023) highlight that only one hundred and eighty-nine research papers on lake MPs were published until 2022.

Multiple factors contribute to freshwater MPs pollution, including urban pressure, industrial pollutants, tourism, improper waste management, illegal dumping, and the degradation of large plastic items. Since freshwater systems are often located in densely populated areas with intense human activity, MPs concentrations in these environments tend to be high (Rodrigues et al., 2018). Additionally, these ecosystems hold great economic value, as they are widely utilized in daily human activities, including drinking water supply, agriculture, fisheries, and recreation (Eerkes-Medrano et al., 2015).

However, the challenges in conducting comprehensive quantitative and qualitative assessments stem from the small size of MPs and the absence of standardized methods for sample collection, processing, characterization, and quantification. This lack of methodological consistency results in insufficient data, hindering reliable risk assessment and limiting spatial and temporal comparisons between studies (Duis and Coors 2016; Löder and Gedts, 2015, Lingshi et al., 2023).

Fish species are increasingly exposed to MPs in freshwater ecosystems, primarily through water, gill absorption, and food ingestion, which can negatively impact various biological processes (Garrido-Gamarro et al., 2020; Wang et al., 2020). Some studies suggest that fish may unintentionally ingest MPs while feeding (Bessa et al., 2018) or through trophic transfer of MPs, from prey to predator (Santana et al., 2017). The first evidence of MP ingestion by freshwater fish was reported by Sanchez et al. (2014), further supporting concerns about their presence in aquatic food webs. As MPs continue to accumulate in freshwater habitats, their potential to disrupt ecosystem stability and threaten fish populations underscores the urgency for further investigation and mitigation strategies. Therefore, the aim of this study is to assess the presence and characteristics of MPs contamination in common bleak (*Alburnus alburnus*, L. 1758) from Gruža Lake using visual identification methods.

Materials and methods

During hydrobiological research conducted in August 2024, samples of wild populations of common bleak (*Alburnus alburnus*, L. 1758) were collected at three localities on Gruža Lake. The common bleak is a gregarious fish species widely distributed across freshwater ecosystems. It uses the surface and mid-water layers for feeding, primarily consuming zooplankton, insect larvae, small invertebrates, and organic detritus. In some cases, it may also ingest phytoplankton and plant particles, depending on food availability. To obtain the samples fish were caught using gill nets (mesh size: 10 mm) following the CPUE (Catch Per Unit Effort) principle. The total length (TL) of each fish was measured to the nearest millimeter, its weight (W) was recorded to the nearest gram, and specimens were placed on ice during transport. Specimens were stored at -20° C until laboratory analyses.

The gastrointestinal tract of the specimens was examined, and a visual inspection of suspect anthropogenic particles was conducted using a NikonSMZ800 stereomicroscope (Nikon Corporation, Tokyo, Japan) equipped with a LeicaFlexacam C3 microscope camera (Leica Microsystems, Wetzlar, Germany), which was used to photograph all suspect particles. The detected particles were counted and classified based on their morphology (size and shape), and optical properties (color and reflective characteristics) according to Lusher et al., 2020.

Results and discussion

The examination of the gastrointestinal (GI) tract content revealed that blue fibers (Figure 1a) were the most frequently detected anthropogenic particles. A type of MPs called fiber is also known as a line or filament if its length is considerably larger than its width. Most studies, especially recent ones, have reported fibers as the dominant type of microplastic (Siddiqa et al. 2025; Rebelein et al., 2021). This type of MP originates from various textiles, including clothing, fishing nets, and geotextiles (Lingshi et al. 2023). Additionally, short black fibers (Figure 1b), transparent films (Figure 1c), and a small number of green pellets (Figure 1d) were identified in our study (Figure 1). Due to the relatively small size of the examined fish, fibers longer than 2.5 mm were not recorded, which is directly related to the size and capacity of the intestinal tract.

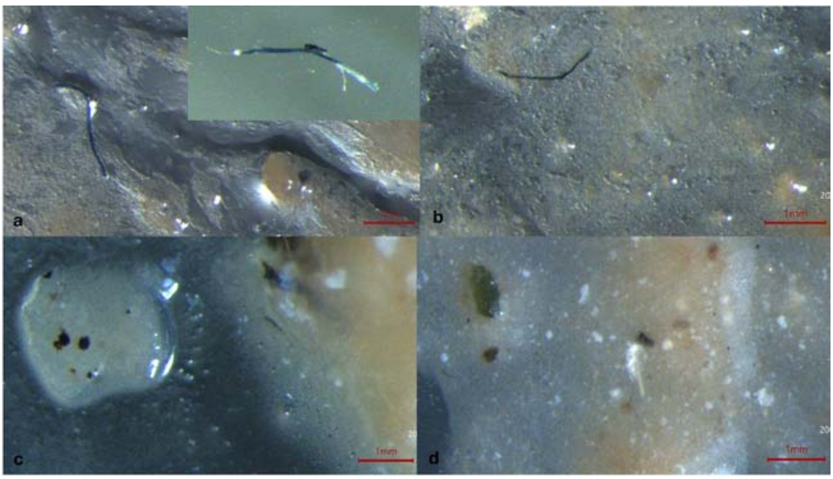

Figure 1. Anthropogenic particles (a) blue fiber; b) black fiber; c) transparent films; d) green pellet)

To trace their pollution sources, most of the existing related studies investigated the morphological characteristics of microplastics. Microplastics were classified based on their morphology (shape, color, and size) according to standard monitoring methods (Lingshi et al., 2023). MPs can have harmful effects on the food chain (Wang et al., 2020), with some researchers suggesting that intentional ingestion may occur when fish mistake MPs for food due to similarities in color or shape (Ory et al., 2017). This risk largely depends on the feeding behavior of the fish and the physical and chemical properties of the

MPs (Roch et al., 2020). The composition of the intestinal content was diverse, and the probability of detecting anthropogenic particles depended on the total amount of ingested material. Filaments and particles were more easily identified and isolated if the content was largely digested. However, when a significant portion of undigested matter was present, certain anthropogenic particles (such as filaments and films) could be masked by the organic material, leading to potential underestimation of their presence. This highlights the necessity of chemical processing of samples (digestion of biological material) to enhance the accuracy of microplastic identification, as well as further analysis using confirmatory techniques such as Fourier-transform infrared (FTIR) or Raman spectroscopy.

Notably, traces of blood were observed in the intestinal tracts of fish containing anthropogenic particles, raising concerns about the potential physiological effects of microplastic ingestion. The extent of microplastic ingestion in fish is largely influenced by plastic pollution in their aquatic environment. Assessing plastic waste distribution in and around water bodies provides valuable insights into potential contamination sources in fish populations.

Due to their poor water circulation and low oxygen levels, lakes have a significantly lower self-purification capacity compared to rivers, making them a major sink for pollutants such as heavy metals, persistent organic pollutants, excess nutrients, and microplastics (Battisti et al., 2020). In the case of Gruža Lake, the findings indicate that plastic waste primarily originates from fishing activities, including abandoned fishing nets, nylon fishing lines, and the use of synthetic bait pellets. This finding indicates that the filaments identified in the fish GI tracts may originate from these materials. Given the widespread recreational fishing in the reservoir, its contribution to plastic pollution is of particular concern.

With the advancement of research, the study of freshwater microplastic pollution has become increasingly important. More than 80% of microplastics found in marine environments originate from terrestrial sources. Additionally, compared to marine ecosystems, freshwater environments are more directly connected to human activities and have a greater impact on daily life (Fan et al., 2022). As essential components of freshwater ecosystems, fish hold significant ecological, economic, and nutritional value worldwide (Pinheiro et al., 2017). Therefore, assessing the risks posed by MPs to freshwater fish populations is vital for ensuring the sustainability of these ecosystems and the resources they provide.

Conclusion

This study highlights the widespread presence of anthropogenic particles in the gastrointestinal tracts of fish, with blue filaments being the most frequently detected. The likelihood of identifying these particles depends on the state of digestion, emphasizing the need for chemical processing to enhance detection accuracy. The observation of blood traces in fish containing microplastics raises concerns about potential physiological effects, warranting further research. Additionally, the results indicate that plastic pollution in the Gruža Lake is largely linked to fishing activities, with nylon fishing lines and nets being the most likely sources of contamination. Given the widespread presence of plastic waste in aquatic ecosystems, continued monitoring and mitigation efforts are essential to assess and reduce its impact on fish and overall environmental health.

Acknowledgement

This research was funded by the Serbian Ministry of Science, Technological Development and Innovation under grant no. 451-03-136/2025-03/ 200122.

References

Ali M. A. A., Khalid A. A., Abd Razak N. I., Mohd Maulana N. S., Roslan N. S., Razmi R. S. B., Wan Ruseli W. M. A., Ibrahim Y. S., Jaafar M., Shahrudin R., Ismail K., Anuar S. T. (2024). A review on the presence of microplastics in environmental matrices within Southeast Asia: elucidating risk information through an analysis of microplastic characteristics such as size, shape, and type. Water Emerging Contaminants & Nanoplastics. Volume (3): 12.

Andrady A. L., Barnes P. W., Bornman J. F., Gouin T., Madronich S., White C. C., Zepp R. G., Jansen M. A. K. (2022). Oxidation and fragmentation of plastics in a changing environment; from UV-radiation to biological degradation. Science of The Total Environment. Volume: (851) 2. 158022.

Aslam H., Ali T., Mortula M. M., Attaelmanan A. G. (2020). Evaluation of microplastics in beach sediments along the coast of Dubai, UAE. Marine Pollution Bulletin. Volume (150): 110739. ISSN 0025-326X.

Aves, A. R., Revell, L. E., Gaw, S., Ruffell, H., Schuddeboom, A., Wotherspoon, N. E., LaRue, M., McDonald, A. J. (2022). First evidence of microplastics in Antarctic snow. The Cryosphere, 16, 2127–2145.

Battisti C., Fanelli G., Filpa A., Cerfolli F. (2020). Giant reed (Arundo donax) wrack as sink for plastic beach litter: first evidence and implication. Marine Pollution Bulletin. Volume (155):111179

Bessa F., Barría P., Neto J. M., Frias J. P. G. L., Otero V., Sobral P., Marques J. C. (2018). Occurrence of microplastics in commercial fish from a natural estuarine environment. Marine Pollution Bulletin, Volume (128): 575-584.

Chen D., Wang P., Liu S., Wang R., Wu Y., Zhu A-X., Deng C. (2024). Global patterns of lake microplastic pollution: Insights from regional human development levels. Science of The Total Environment. Volume (954). 176620, ISSN 0048-9697

David K. A. B., Galgani F., Thompson R. C., Barlaz M. (2009). Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B. Volume (364): 1985–1998.

Dris R., Beaurepaire M., Bouzid N., Stratmann C., Nguyen M. T., Bordignon F., Gasperi J., Tassin B. (2024). Chapter 3 - Sampling and analyzing microplastics in rivers: What methods are being used after a decade of research? , Editor(s): Eddy Y. Zeng, Microplastic Contamination in Aquatic Environments (Second Edition), Elsevier, Pages 65-91, ISBN 9780443153327.

Duis K., Coors A. (2016). Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environmental Sciences Europe. Volume (28): 2

Eerkes-Medrano D., Thompson R. C., Aldridge D. C. (2015). Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Research. Volume (75): 63-82.

Fan J., Zou L., Duan T., Qin L., Qi Z., Sun J. (2022). Occurrence and distribution of microplastics in surface water and sediments in China’s inland water systems: a critical review. Journal of Cleaner Production. Volume (331):129968

Garrido-Gamarro E., Ryder J., Elvevoll E. O., Olsen R. L. (2020). Microplastics in Fish and Shellfish – A Threat to Seafood Safety? Journal of Aquatic Food Product Technology. Volume (29) 4: 417–425.

Geyer R., Jambeck J. R., Lavender K. (2017). Law Production, use, and fate of all plastics ever made. Science Advances. Volume (3):1700782.

Kögel T, Hamilton B. M., Granberg M. E., Provencher J., Hammer S., Gomiero A., Magnusson K., Lusher A. (2023). Current efforts on microplastic monitoring in Arctic fish and how to proceed. Arctic Science. Volume **9**(2): 266-283.

Lambert S., Scherer C., Wagner M. (2017). Ecotoxicity testing of microplastics: Considering the heterogeneity of physicochemical properties. Integrated Environmental Assessment and Management, Volume (13): 3. 470–475.

Lingshi Y., Li D., Xiaofeng W., Danlian H., Ruihao X., Zeyu W., Hongyu S., Jiali H., Guangfu W., Jiaxi T., Yuantai K. (2023). Occurrence and Effects of Microplastics in Lake Ecosystems: Particular Focus on Migration in Water and Food Chains. Reviews of Environmental Contamination and Toxicologyformerly. Volume (261) 11.

Löder M.G.L., Gerdts, G. (2015). Methodology Used for the Detection and Identification of Microplastics—A Critical Appraisal. Marine Anthropogenic Litter (eds. Bargeman, M., Gutow, G. and Klages, M.). Heidelbderg, Germany. Springer Open. 201-227.

Lusher A. L., Brate I. L. N., Munno K., Hurley R. R., Welden N. A. (2020). Is It or Isn`t It: The Importance of Visual Classification in Microplastic Characterization. Applied Spectroscopy, Special Issue: Microplastics. Volume 0: 1-15

Maurizi L., Simon-Sánchez L., Vianello A., Nielsen A. H., Vollertsen J. (2024). Every breath you take: High concentration of breathable microplastics in indoor environments. Chemosphere, Volume (361): 142553, ISSN 0045-6535.

Maheswaran B., Karmegam N., Al-Ansari M., Subbaiya R., Al-Humaid L., Raj J. S., Govarthanan M. (2022). Assessment, characterization, and quantification of microplastics from river sediments. Chemosphere. Volume (298): 134268,

Nuamah F., Tulashie S. K., Debrah J. S., Pèlèbè R. O. E. (2023). Microplastics in the Gulf of Guinea: An analysis of concentrations and distribution in sediments, gills, and guts of fish collected off the coast of Ghana. Environmental Research. Volume (234): 116567, ISSN 0013-9351.

Ory N. C., Sobral P., Ferreira J. L., Thiel M. (2017). Amberstripe scad Decapterus muroadsi (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. Science of The Total Environment. Volume (586): 430-437.

Pinheiro C., Oliveira U., Vieira M. (2017). Occurrence and Impacts of Microplastics in Freshwater Fish. Journal of Aquaculture & Marine Biology. Volume (5): 6.

Plastics Europe (2019) Plastics—The Facts 2019. https://plasticseurope.org/knowledge-hub/plastics-the-facts-2019/

Rebelein A., Int-Veen I., Kammann U., Scharsack J. P. (2021). Microplastic fibers — Underestimated threat to aquatic organisms?. Science of The Total Environment. Volume (777): 146045.

Ritchie M. W., Cheslock A., Bourdages M. P. T., Hamilton B. M., Provencher J. F., Allison J. E., MacMillan H. A. (2023). Quantifying microplastic ingestion, degradation and excretion in insects using fluorescent plastics. Conservation Physiology Volume:11(1).

Roch S., Friedrich C., Brinker, A. (2020). Uptake routes of microplastics in fishes: practical and theoretical approaches to test existing theories. Scientific Reports. Volume (10): 3896

Rodrigues M.O., Abrantes N., Gonçalves F.J.M., Nogueira H., Marques J.C., Gonçalves A.M.M. (2018). Spatial and temporal distribution of microplastics in water and sediments of a freshwater system (Antuã River, Portugal). Science of The Total Environment. Volume (633): 1549-1559.

Salerno M., Berlino M., Mangano M. C., Sarà G. (2021). Microplastics and the functional traits of fishes: a global meta-analysis. Global Change Biology. Volume 27(12). 2645-2655.

Sanchez W., Bender C., Porcher J. M. (2014). Wild gudgeons (Gobio gobio) from French rivers are contaminated by microplastics: preliminary study and first evidence. Environmental Research. Volume (128): 98-100.

Santana M.F.M., Moreira F.T., Turra A. (2017). Trophic transference of microplastics under a low exposure scenario: Insights on the likelihood of particle cascading along marine food-webs. Marine Pollution Bulletin. Volume (121): 154-159.

Siddiqa S., Gautam S., Eti S. A., Khatun F., Rahman M. M., Solayman H. M., Aziz A. A., Khan B. (2025). Characterization, distribution, and risk assessment of microplastic in fish and sediment from the longest river of Bangladesh. Water Environment Research. Volume 97(2), e70029.

Singh J., Yadav B. K., Schneidewind U., Krause S. (2024). Microplastics pollution in inland aquatic ecosystems of India with a global perspective on sources, composition, and spatial distribution. Journal of Hydrology: Regional Studies. Volume (53) 101798.

Taha Z. D., Amin R. Md., Anuar S.T., Nasser A. A. A., Sohaimi E. S. (2021). Microplastics in seawater and zooplankton: A case study from Terengganu estuary and offshore waters, Malaysia. Science of The Total Environment. Volume (786):147466, ISSN 0048-9697.

Wang T., Wang L., Chen Q., Kalogerakis N., Ji R., Ma Y. (2020). Interactions between microplastics and organic pollutants: Effects on toxicity, bioaccumulation, degradation, and transport. Science of The Total Environment. Volume (748): 142427.

Zhang Y., Gao T., Kang S., Allen S., Luo X., Allen D. (2021). Microplastics in glaciers of the Tibetan Plateau: Evidence for the long-range transport of microplastics. Science of The Total Environment. Volume (758). 143634, ISSN 0048-9697