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Burbot (*Lota lota*) as a bioindicator of microplastic pollution in the Tisza River: Multi-tissue contamination, polymer characterisation, and implications for ecological and human-health risks

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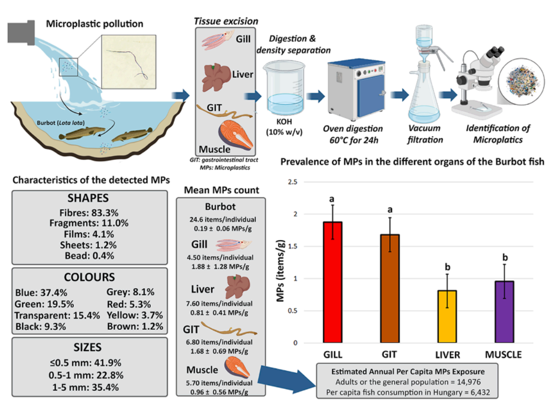
HIGHLIGHTS

- This study provides the first evidence of microplastic (MP) contamination in burbot.
- All sampled fish contained MPs, averaging 24.6 particles per individual.
- Gills (1.88 MPs/g) and gastrointestinal tracts (1.68 MPs/g) had the highest MP loads.
- Most MPs were small (41.9 %), blue (37.4 %), fibre-shaped (83.3 %), and textile-derived.
- Results highlight the urgent need for stricter waste management policies.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastic (MP) pollution poses a growing threat to freshwater ecosystems and human health, yet knowledge of its occurrence in European rivers remains limited. This study provides the first evidence of MP contamination in burbot (Lota lota) from the Hungarian upper Tisza River. MPs were detected in all sampled fish (n = 10), with an average of 24.6 ± 7.46 particles per individual. Most particles were fibres (83.3 %), predominantly blue and generally < 500 µm in diameter. A Kruskal–Wallis analysis showed significant differences in MP retention across tissues ($p < 0.05$), with gills (1.53 MPs g^{-1}) and the gastrointestinal tract (GIT; 1.54 MPs g^{-1}) containing higher

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loads than liver (0.89 MPs g^{-1}) and muscle (1.07 MPs g^{-1}). Correlation analysis indicated non-significant positive associations ($p \geq 0.05$) between total MP load and retention in the GIT ($r = 0.41$), liver ($r = 0.58$), and muscle ($r = 0.63$), but a negative relationship with gills ($r = -0.56$). Raman spectroscopy confirmed 68 particles representing 17 polymer and copolymer types, grouped into three categories: anthropogenically modified cellulosic fibres (ACF), textile-derived MPs, and petroleum-derived MPs. Textile-associated materials, including ACF, PET–cotton blends, and indigo-dyed polyurethane, predominated, indicating strong inputs from domestic laundering and urban effluent. Estimated human exposure through burbot consumption ranged from 14.4 MPs/week in infants to 320.54 MPs/week in European adults (123.6 MPs/week in Hungary), highlighting a potential dietary exposure pathway. These findings show that inadequate upstream waste management drives contamination and provide a critical baseline for monitoring microplastic pollution in Central European freshwater systems. Given its ecological traits and tissue-specific retention patterns, burbot represents a valuable bioindicator for long-term riverine MP surveillance.

1. Introduction

Microplastic (MP) pollution, characterised by plastic particles smaller than 5 mm, has become a significant global environmental concern due to its persistence and potential hazards to ecosystems and human health [1]. These particles originate from primary sources, such as microbeads in cosmetics, or from secondary sources, including the breakdown of larger plastic debris. MPs are pervasive in aquatic environments, where they cause physical damage, introduce associated chemical contaminants, and accumulate in tissues of exposed organisms, with negative consequences for biodiversity and food security [2,3]. While marine MP contamination has received considerable scientific attention, research on freshwater ecosystems, which serve as conduits for land-based plastic waste into the oceans, remains relatively limited, especially in Central and Eastern European regions [4,5]. In addition, rivers play a pivotal role in this transport, with 122 heavily contaminated rivers responsible for over 90 % of the 1.15–2.41 million tonnes of plastic debris entering oceans each year [6].

In Europe, rivers such as the Danube, Rhine, and Seine exhibit substantial variability in MP loads, with concentrations ranging from negligible levels to 187,000 particles per cubic meter (items/m^3) in surface waters, and up to 72,400 particles per kilogram (items/kg) in sediments [2,7]. This highlights the widespread nature of MP contamination, even in regions with advanced waste management systems. Countries in Eastern Europe, such as Hungary, face additional challenges due to a high level of unmanaged plastic waste, which is a growing issue exacerbated by limited wastewater treatment infrastructure [8]. For instance, in the Upper Tisza catchment in Ukraine, only 1.5 % of rural households have access to wastewater treatment, resulting in untreated effluents containing textile-derived microfibres from household washing machines, fuelled by the widespread use of inexpensive synthetic fabrics [8–10].

As a result, the Hungarian stretch of the Tisza River exhibits substantial MP pollution, with surface water concentrations ranging from 4 to 63 items/m^3 and sediment loads from 528 to 8067 items/kg, driven by wastewater discharges, agricultural runoff, and urban activities [5, 9]. Additional stressors include Hungary's growing plastic packaging waste (increasing from 21 kg per capita in 2010–35 kg in 2019), relatively low plastic packaging recycling rates (22 %), and a high percentage of plastic waste ending up in landfills (57 %) [11,12]. These factors collectively highlight the urgent need for in-depth investigations into the ecological impacts of plastic pollution in river systems, such as the Tisza.

Fish, particularly those at higher trophic levels, serve as effective bioindicators of MP contamination because they tend to ingest plastic particles directly or via prey [13]. The burbot (*Lota lota* Linnaeus, 1758), a benthic and demersal predator native to the Tisza River, is well-suited as a sentinel species for assessing microplastic contamination. Its strictly bottom-oriented lifestyle and nocturnal foraging on macroinvertebrates and small fish can result in frequent exposure to microplastics originating both from the sediment-water interface and via trophic transfer [14,15]. Furthermore, as a long-lived species that integrates environmental stressors over time, burbot can provide a reliable representation of local contamination patterns. Moreover, its recognised role in water quality assessments under the EU Water Framework Directive [16–18], combined with its status as a freshwater species attracting growing aquaculture interest due to its achievable nutritional quality, high protein content and omega-3 fatty acids under rearing conditions [19,20], highlights its relevance for evaluating both ecological and human-health risks associated with microplastic pollution

Despite the compelling rationale for studying MPs ingestion in burbot, no prior research has investigated this species. While MP pollution has been documented in freshwater systems and various fish species globally [21–24], data on MP contamination in the Hungarian section of the Tisza River remain scarce. This paucity of information represents a significant gap in understanding the species-specific and regional impacts of MPs in freshwater ecosystems. Although a previous study in the Tisza River reported MP retention in freshwater mussels (*Unio tumidus* and *Unio crassus*), with particle abundances ranging from 2.7 to 8.3 per individual [25], no research has yet explored MP contamination in fish from this region. The present study's purpose is to address this critical gap by providing the first assessment of MP ingestion in burbot from the Hungarian Tisza River and offering novel insights into the extent and implications of MP pollution in this ecologically significant freshwater system. To achieve this purpose, this research aimed to (1) quantify MP burden in burbot from the Hungarian section of the Tisza River, (2) identify the major polymer and pigment types associated with ingested MPs, (3) infer potential pollution sources, and (4) evaluate ecological implications of MP retention in a sentinel fish species. Using advanced Raman spectroscopy, the study establishes a crucial baseline for future biomonitoring efforts and offers valuable insights into the dynamics of MP pollution in Central European freshwater ecosystems.

2. Materials and methods

2.1. Study area

The Tisza River, a key fluvial system in Central and Eastern Europe, is the largest tributary of the Danube and drains approximately 157,200 km^2 across Ukraine, Romania, Slovakia, Hungary, and Serbia [9]. The river originates in the Carpathian Mountains, where annual precipitation ranges between 1000 and 1400 mm in the upstream sub-basins of Ukraine, Romania, and Slovakia [26]. As it flows into the low-lying Carpathian Plains, the Hungarian section, its longest stretch, plays a pivotal role in regional hydrology and ecology [27]. The upper reaches of the Tisza, particularly in Ukraine, experience significant pollution pressures, primarily due to poorly managed solid waste, direct discharge of untreated wastewater, and diffuse inputs from agricultural and mining activities [28]. These pollutants, essentially comprising plastics and inorganic debris, are often deposited along riverbanks and mobilised downstream during flood events, contributing to MP accumulation in the Hungarian segment of the river [27].

Plastic pollution in this watercourse has intensified considerably in recent years. For instance, a cleanup operation in 2017 removed around 90 tonnes (3084 m^3) of plastic waste, predominantly in the upper Hungarian section near the Ukrainian border [29]. By 2019, the volume of plastic debris in the same area was estimated at roughly 10,000 tonnes, indicating a sharp increase in pollution inputs [30]. Recent data from 2021 to 2022 revealed a 17 % increase in MP flux, with peak concentrations recorded in both areas near the Hungarian-Ukrainian border and in Serbia, where waste management infrastructure is insufficient [5]. To investigate microplastic pollution at this critical juncture, fish specimens were collected at Tiszabecs, located at the Hungarian-Ukrainian border, where the Tisza River enters Hungary's Northern Great Plain (Fig. 1), a strategic site for monitoring transboundary pollution from upstream sources.

2.2. Fish Sampling and Preparation

The burbot, a benthic and piscivorous species, was chosen as a focal organism for MP analysis owing to its ecological relevance, increasing significance in aquaculture, and dietary value to humans. Its demersal lifestyle and feeding habits render it particularly susceptible to MP ingestion, as riverbeds are standard sinks for MP debris and associated contaminants [31]. A total of ten adult burbot fish were captured from the Tisza River at Tiszabecs using an electric fishing device (Hans Grassl EL64 II GI, DC, 300/600 V max. 7 kW, Hans Grassl GmbH, Germany; permission number: HaGF/68/2021). The fish were transported in metallic containers with aerated river water to the laboratory. Upon arrival, individuals were rinsed with deionised water to eliminate any external particles. Morphometric parameters of each specimen, including the standard length (SL), were recorded to the nearest 0.1 mm using calibrated measuring instruments, and the total weight (W) was measured to the nearest 0.01 g with a portable precision digital scale (Ohaus Scout™ SPX, OHAUS Corporation, Parsippany, NJ, USA). After the measurements, the fish were sacrificed immediately by spinal severance and stored at −18°C until sample processing. Before dissection, specimens were thawed at ambient temperature, and any remaining surface blood or debris was removed with deionised water. Dissections were performed under sterile conditions using stainless steel instruments to prevent sample contamination. Internal organs, including the gastrointestinal tract (GIT) and its contents, gills, liver, and dorsal muscle tissue samples, were carefully excised, weighed using an analytical balance (Precisa 240 A, Switzerland), and placed in individual, pre-cleaned glass beakers or sealed glass containers covered with aluminium foil to minimise exposure to airborne MPs [32], then processed immediately for chemical digestion and microplastic characterisation.

2.3. Tissue digestion and microplastic filtration

To isolate MPs from burbot tissues, digestion procedures were adapted from established methods [23]. Whole tissues, including the gastrointestinal tract, liver, and gills, were excised, weighed, and transferred to pre-cleaned glass beakers. For the muscle analysis, a standardised 5-g portion of dorsal muscle was subsampled from each fish, following widely applied approaches in MP biomonitoring of fish and shellfish [32–35]. This consistent subsampling ensured comparable tissue masses across individuals and enabled MP concentrations to be reliably expressed as MPs per gram of muscle tissue. All samples were immersed in a 10 % (w/v) KOH solution at a 1:3 tissue-to-digestant ratio, ensuring complete submersion before digestion. Beakers were sealed with aluminium foil to limit airborne contamination and incubated at 60°C for 24 h. The samples were gently agitated every 12 h to support thorough tissue breakdown. Given the high lipid content of liver tissue, ethanol (EtOH; 97 % purity) was added at a 1:4 ratio (digestate + KOH:EtOH), following protocols outlined by Dawson et al. [36] and Mancuso et al. [37] to minimise saponification. Procedural blanks containing filtered Milli-Q water were processed alongside the experimental samples to monitor environmental contamination. After digestion, a density separation step was performed as previously described [23] by adding saturated sodium iodide (NaI, density 1.8 g/cm^3) in a 1:2 ratio (digestate:NaI). The mixtures were vigorously agitated, resealed, and left to settle overnight. Afterwards, the digestates were processed by vacuum filtration using Whatman GF/A glass microfibre filters (47 mm diameter, 1.6 µm pore size). In samples where digestion was complete or residual material was minimal, the entire digestate (liquid and remaining solids) was filtered directly. For samples containing a greater amount of undigested material, the supernatant was first vacuum

Fig. 1. Map of the sampling site with the Tiszabecs point marked in red. HU: Hungary; UA: Ukraine

filtered, after which the remaining solids, including gill rakers or other refractory gastrointestinal residues, were carefully collected onto a separate GF/A filter. All retained materials were systematically examined under the stereomicroscope to ensure complete recovery of microplastics potentially trapped within the precipitate. Between samples, the filtration apparatus was rinsed thoroughly with filtered Milli-Q water to minimise cross-contamination. Following filtration, filters were transferred into clean, labelled Petri dishes, covered with aluminium foil, and air-dried at room temperature for 24 h before being sealed and stored for subsequent analysis.

2.4. Microplastic identification and morphological classification

Photographs of the recovered MPs were taken, and measurements were performed using an Olympus SZX16 stereomicroscope equipped with an Olympus UC90 digital camera and operated via the Evident Olympus cellSens Entry imaging software (Version 4.2; CS-EN-V4.2). The particles retained on each filter were visually inspected, counted, and sorted by morphological category (fibres, fragments, films, microspheres, and foams) as well as by colour (e.g., black, blue, red, green, white, transparent, yellow, and grey), following procedures by Bessa *et al.* [38] and Rodrigues *et al.* [39]. The largest cross-sectional dimension of each particle was measured in situ and binned into six size classes: $\leq$ 0.5 mm, 0.5–1 mm, 1–2 mm, 2–3 mm, 3–4 mm, and 4–5 mm. To ensure traceability during subsequent analyses, individual particles were transferred onto gridded filter papers and labelled accordingly for Raman spectroscopy.

2.5. Chemical Characterisation of Microplastics

To determine polymer composition, a subset of microplastic particles was selected for analysis using a Horiba LabRam HR Evolution Confocal Raman spectroscope (HORIBA Scientific). Selection was designed to include a range of particle sizes, colours, and morphologies to ensure representativeness. Raman spectroscopy was performed on 68 microplastic particles, representing 27.6 % (68/246) of the total number of MPs recovered. This analytical coverage is in line with, and in several cases exceeds, the proportion of particles subjected to polymer identification in comparable organism-based MP studies [e.g., 21–24]. Particles selected for Raman analysis were not chosen based on predefined characteristics such as colour, size, or morphology; instead, all particles recovered from the first four fish samples were analysed. As a result, the subset encompassed all primary colour and shape categories present in the overall dataset and preserved their naturally occurring proportional distribution. While specific particle categories occurred at low frequencies, reflecting the inherently uneven distribution of MPs, all available particles belonging to such rare categories were included where possible. Consequently, the Raman-analysed subset provides a robust and representative overview of the polymer composition of MPs detected in burbot tissues, while acknowledging that rare particle types may limit detailed statistical interpretation.

The system was equipped with a cooled CCD detector and operated using a 532 nm diode laser. To capture the spectra, a 600 lines/mm diffraction grating was applied, providing a peak position accuracy within $\pm$1.5 cm^{-1} . The laser beam was directed onto the sample surface using a 50x objective lens, delivering an output intensity of approximately one mW cm^{-2}. Each measurement was taken over a 10-second acquisition period and repeated 10 times (10 accumulations) to ensure signal clarity. After data collection, the spectra were baseline-corrected before further analysis. Post-acquisition spectral processing involved polynomial baseline correction in LabSpec 6 Spectroscopy Suite Software (HORIBA Scientific) to enhance clarity and reduce background noise. Polymer identification was achieved using Spectragryph v1.2.16 [40], cross-referencing with spectral libraries containing synthetic polymers, pigments, minerals, and organic materials. Additional confirmation was conducted using OpenSpecy software [41]. A match confidence threshold of $\geq$ 70 % or $\geq$ 0.7 probability was applied to validate identifications.

2.6. Quality assurance and quality control (QA/QC)

Robust QA/QC procedures were employed throughout sampling and analysis to prevent false positives due to contamination, in line with best practices in MP research [9,42,43]. All personnel wore nitrile gloves and plain white cotton laboratory coats, which were selected to reduce synthetic fibre contamination [44]. All chemicals, including KOH, EtOH, and NaI, were pre-filtered through Whatman 8 μm filters. All dissections, sample preparation, and filtration were conducted in a controlled clean-room environment or within a horizontal laminar flow cabinet to minimise airborne and external contamination. Laboratory workspaces and tools were thoroughly cleaned with 70 % ethanol, and only glass or stainless-steel instruments, pre-rinsed with Milli-Q water and oven-dried, were used for sample handling. This cleaning protocol was applied consistently to prevent cross-contamination between fish specimens and among the different tissues processed. Procedural blanks and airborne deposition controls were run concurrently with samples at each stage of the process to monitor background contamination [45]. A total of 52 suspected MPs were detected in procedural blanks during laboratory processing and analysis. To ensure conservative and accurate estimates, particles in the blanks exhibiting characteristics similar to those in the samples (shape, size, and colour) were excluded from the final dataset. Notably, nearly all cotton fibres identified in the samples were distinctly coloured (e.g., blue, green, or red).

In contrast, all laboratory coats were plain white cotton, confirming that these fibres did not originate from laboratory clothing. While a colour mismatch strongly supports an environmental origin, the possibility of undetected laboratory contamination cannot be entirely ruled out. Furthermore, cotton fibres confirmed by Raman spectroscopy were evaluated for anthropogenic modification: only fibres showing clear spectral evidence of textile dyes (e.g., indigo) or chemical treatments were classified as anthropogenic and included in the dataset for polymer identification and analysis. Untreated natural fibres lacking such chemical signatures were excluded, ensuring accurate discrimination between environmental microplastics and unmodified cellulose.

2.7. Estimation of human exposure to MPs through burbot consumption

To estimate human exposure to MPs through consumption of burbot muscle tissue, we applied a deterministic exposure assessment framework adapted from Barboza *et al.* [46] and later extended by Matias et al. [47]. First, the MP concentration in dorsal muscle tissue (MPs/g) was calculated by dividing the total number of MPs detected in each sample by its corresponding muscle mass. Age-specific weekly fish consumption values (g/week) were obtained from the European Food Safety Authority's Scientific Opinion on fish and seafood consumption [48], while Hungary's national average annual fish consumption, estimated at 6.7 kg per person [49], was used to contextualise exposure over a longer time frame, approximating 129 g per week. Standard body-weight assumptions for different demographic groups were sourced from the EFSA Scientific Committee guidance (5 kg for infants, 12 kg for toddlers, 23.1 kg for older children, 52.4 kg for adolescents, and 70 kg for adults) [50]. Weekly and yearly MP intake were then estimated by multiplying the MP concentration of the burbot muscle (MPs/g) by the corresponding weekly fish consumption values (g/week). To improve toxicological relevance, exposure was normalised by body weight and presented as particles/kg bw/week and particles/kg bw/year. This assessment included infants, toddlers, older children, adolescents, adults, and Hungarian consumers to provide a comprehensive evaluation of potential MP exposure across different population groups. Because burbot-specific consumption data were unavailable, EFSA's general fish consumption values were used as a proxy. These values may not fully represent burbot intake patterns; however, they provide the most reliable and standardised consumption estimates available. These estimates provide preliminary insight into potential MP ingestion through local fish consumption and contribute to understanding the public health implications of MP contamination in freshwater fish from the Tisza River. Their use offers a consistent and conservative basis for modelling MP exposure while acknowledging this limitation.

$$\begin{aligned}&\textit{Human MP intake per week}(\textit{MP items}/\textit{week})\\&\quad= \textit{Average MP item in the muscles}(\textit{MPs items}/g)\\&\qquad\times \textit{Recommended fish food intake per week}(g)\end{aligned} \tag{1}$$

$$\begin{aligned}&\textit{Human MP intake per year}(\textit{MP items}/\textit{year})\\&\quad= \textit{Average MP item in the muscles}(\textit{MPs items}/g)\\&\qquad\times \textit{Recommended fish food intake per year}(g)\\&\qquad\times \textit{Number of week per year}(52)\end{aligned} \tag{2}$$

2.8. Statistical analyses

Descriptive statistics were used to summarise the morphometric characteristics of burbot and to characterise the microplastic (MP) particles recovered from each tissue. MPs were classified by morphological type (fibres, fragments, films, microspheres, foams), colour (e.g., black, blue, red, green, yellow, white, grey, transparent), and size class ($\leq$ 0.5 mm, 0.5–1 mm, 1–2 mm, 2–3 mm, 3–4 mm, and 4–5 mm). MP retention in the GIT, gills, liver, and muscle was standardised to particles per gram of tissue. All statistical analyses were performed in Python [51] and R (version 4.3.3) [52]. Assumptions of normality and homogeneity of variance were evaluated using the Shapiro-Wilk and Levene's tests, respectively. Because at least one tissue group violated normality assumptions (gills: $W = 0.71$, $p = 0.001$), and sample sizes were modest ($n = 10$ per tissue), tissue-level comparisons were conducted using a non-parametric Kruskal-Wallis test. When significant differences were detected, Dunn's post-hoc test with Benjamini–Hochberg correction was applied for pairwise comparisons. Correlations between MP abundance and organ weights were assessed using Pearson's correlation coefficient, with significance set at $p < 0.05$. Data visualisations were generated using ggpubr to support interpretation.

3. Results

3.1. Morphometric profiles and microplastic load in Burbot

Morphometric data, including total length and individual organ weights (GIT, gills, liver, and dorsal muscle), are summarised in Table 1. On average, burbot weighed 132.49 ± 20.77 g (Table 2), with a total length of 265.0 ± 15.32 mm. Mean organ weights were as follows: GIT – 4.08 ± 0.42 g, gills – 2.41 ± 0.23 g, liver – 9.43 ± 0.80 g, and dorsal muscle – 6.02 ± 0.81 g (Table 1).

3.2. Microplastic abundance across tissues

Microplastics were detected in all ten burbot examined (100 % prevalence), yielding a total of 246 particles across the four tissues. This equates to an average of 24.6 ± 7.46 MPs per individual and 188.48 ± 58.44 MPs per kilogram of body weight (Table 2). In absolute counts, the liver contained the highest number of particles (76 MPs; 30.9 %), followed by the GIT (68 MPs; 27.6 %), dorsal muscle (57 MPs; 23.2 %), and gills (45 MPs; 18.3 %). The mean unstandardised MP counts per tissue were 7.6 ± 2.3 MPs (liver), 5.7 ± 1.8 (muscle), 4.5 ± 1.5 (gills), and 2.6 ± 0.9 (GIT).

When standardised to tissue weight, median MP concentrations were highest in the GIT (1.54 MPs/g) and gills (1.53 MPs/g), intermediate in muscle (1.07 MPs/g), and lowest in liver (0.89 MPs/g) (Fig. 2). Shapiro–Wilk tests indicated non-normality in gill samples ($W = 0.71$, $p = 0.001$), whereas other tissues did not deviate significantly from normality. Levene's test confirmed homogeneity of variances ($p = 0.63$). Given the non-parametric distribution, a Kruskal–Wallis test was used to compare the tissues, revealing significant differences in MP retention ($H = 12.95$, $p = 0.0047$, $DF = 3$). Dunn's post-hoc analysis (Benjamini–Hochberg adjusted) showed that liver tissue contained significantly fewer MPs/g (0.89) than both the GIT (1.54 MPs g^{-1}) and gills (1.53 MPs g^{-1}) (adjusted $p = 0.013$ for each). Muscle tissue (1.07 MPs g^{-1}) did not differ significantly from either GIT/gills (adjusted $p = 0.056$) or liver (adjusted $p = 0.53$). These results indicate that MP retention follows the pattern GIT ≈ Gills > Muscles > Liver, with gill and GIT tissues serving as the primary sites of microplastic retention, consistent with their physiological roles in ingestion and respiration.

Pearson correlation analysis was used to explore potential linear relationships between organ weights and MP quantities. Although several correlations appeared moderate in magnitude: GIT ($r = 0.41$, $p = 0.245$), liver ($r = 0.58$, $p = 0.080$), and muscle ($r = 0.63$, $p = 0.052$), with a moderate negative correlation for gills ($r = -0.56$, $p = 0.089$), none were statistically significant ($p \geq 0.05$). These findings indicate that, within the present sample, there is no evidence for a meaningful linear association between tissue mass and MP load (Fig. 3).

3.3. Morphological characteristics of microplastics

MPs were classified based on physical attributes, shape, colour, and size within each tissue type (Figs. 4–9). The majority of particles were fibres (83.3 %, $n = 205$), followed by fragments (11.0 %, $n = 27$), films (4.1 %, $n = 10$), sheets (1.2 %, $n = 3$), and a single bead (0.4 %, $n = 1$) (Fig. 4). Fibre prevalence was consistent across tissues, with the highest in the dorsal muscle (92.98 %) and the lowest in the gills (68.89 %) (Fig. 5). Fragments were most frequent in the GIT (16.18 %, $n = 11$), while films and sheets were sporadically distributed. Notably, beads were detected only in the muscle (1.75 %, $n = 1$).

Colour analysis revealed blue MPs as the most dominant (37.4 %, $n = 92$), followed by green (19.5 %, $n = 48$) and transparent particles (15.4 %, $n = 38$) (Figs. 6 and 7). Blue particles ranged from 32.89 % in the liver to 42.11 % in the dorsal muscle. Green particles were most prevalent in the liver (27.63 %) and least in the gills (13.33 %). Transparent MPs were highest in the GIT (22.06 %) but least common in liver samples (7.89 %). Black particles appeared more frequently in the GIT (13.24 %) and liver (10.53 %) than in gills (8.89 %). Colours such as brown, grey, red, and yellow occurred in low proportions, with brown completely absent from the dorsal muscle.

Across all tissues combined, MPs were strongly dominated by the tiniest particles (≤0.5 mm), which accounted for 41.9 % of all items detected, followed by the 0.5–1 mm and 1–2 mm classes (22.8 % and 24.8 %, respectively). Larger particles (>2 mm) were comparatively rare, collectively representing < 11 % of all MPs recovered. When examined by tissue type, a consistent pattern emerged: the smallest

Table 1
Morphometric measurements of burbot (*Lota lota*) and corresponding tissue masses used for microplastic analysis.

Species	ATL (Range) mm	ASL (Range) mm	GIT_W (Range) g	GILL_W (Range) g	LIV_W (Range) g	MUS_W (Range) g
Burbot	265 ± 15.52 (250–294)	237 ± 15.29 (220–266)	4.08 ± 0.42 (3.39 – 4.92)	2.41 ± 0.23 (2.09 – 2.89)	9.43 ± 0.80 (6.4 – 10.1)	6.02 ± 0.81 (4.50 – 7.13)

Values represent mean ± SD, with ranges shown in parentheses. ATL: Average Total Length; ASL: Average Standard Length; GIT_W: Mean Gastrointestinal Tract (GIT) Weight; GILL_W: Mean Gill Weight; LIV_W: Mean Liver (LIV) Weight; MUS_W: Mean Muscle (MUS) Weight.

Table 2
An abundance of microplastic particles was detected in burbot tissues (n = 10).

FISH ID	Body Weight (g)	Tissue MPs No				MPs/Ind.	MPs/BW(kg)
		GIT	Gill	Muscle	Liver		
Burbot_1	132.49	5	4	6	3	18	135.86
Burbot_2	173.14	8	3	1	8	20	115.51
Burbot_3	139.96	9	2	2	5	18	128.61
Burbot_4	150.49	10	3	11	10	34	225.93
Burbot_5	116.56	5	3	11	14	33	283.12
Burbot_6	103.32	6	5	9	2	22	212.93
Burbot_7	119.2	6	6	2	3	17	142.62
Burbot_8	123	4	11	6	9	30	243.90
Burbot_9	148.82	12	4	6	13	35	235.18
Burbot_10	117.94	3	4	3	9	19	161.10
Total	**1324.9**	**68 (27.64 %)**	**45 (18.29 %)**	**57 (23.17 %)**	**76 (30.89 %)**	**246**	
Mean	**132.49**	**6.80**	**4.50**	**5.70**	**7.60**	**24.60**	**188.48**
SD	**20.77**	**2.86**	**2.55**	**3.71**	**4.22**	**7.46**	**58.44**
Mean MP per g BW (MPs/g)		1.68 ± 0.69	1.88 ± 1.28	0.96 ± 0.56	0.81 ± 0.41		

*MP counts are reported per tissue (GIT, gill, muscle, liver), per individual fish, and normalised to body weight (MPs/kg BW); Gastrointestinal Tract: GIT; Ind.: Individual; Mean MP/g of tissues ± standard error of mean. BW: Body Weight. Tissue-specific median MP concentrations (MPs/g) for each organ are presented in Fig. 2.

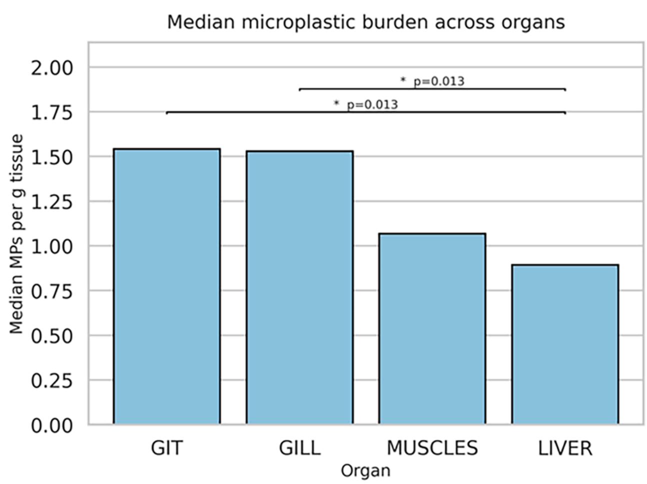

Fig. 2. Abundance of suspected microplastics (MPs g^{-1}) across burbot tissues. GIT = gastrointestinal tract. Overall tissue differences were assessed using a Kruskal–Wallis test ($H = 12.95$, $p = 0.0047$, df = 3), followed by pairwise Dunn's tests with Bonferroni correction. Bars with linking lines are significantly different ($p < 0.05$).

particles remained the most prevalent in all tissues, though their proportional distribution varied (Fig. 9). MPs ≤ 0.5 mm constituted the highest proportion in the gills (46.7 %), followed by the liver (42.1 %), muscles (40.4 %), and GIT (39.7 %). Particles in the 0.5–1 mm and 1–2 mm classes also occurred frequently across tissues, together accounting for 42.3 %-53 % of MPs, depending on tissue type. In contrast, larger MPs (>2 mm) were least represented across all tissues but showed slightly higher proportions in gills (8.9 %) and liver (10.5 %) compared with the GIT (4.4 %) and muscles (3.5 %). This distribution suggests that while all tissues preferentially accumulate smaller particles, the GIT is more likely to retain a wider size range, including relatively larger MPs. The stereomicroscope images of identified suspected MPs in various tissues of the burbot are presented in Fig. 10.

3.4. Polymer Composition and Tissue-Specific Distribution of Identified Particles

Raman spectroscopy confirmed the polymer identity of 68 particles, representing 17 distinct polymers and copolymers (Fig. 11). All Raman-confirmed particles were analysed and categorised to distinguish natural fibres from anthropogenically modified materials. All cellulosic fibres showed clear evidence of human-derived alteration, such as dyeing, coating, blending with synthetics, or indigo blue pigmentation, indicating that none originated from unmodified natural sources. Based on this, the dataset was grouped into three polymer categories: (1) anthropogenically modified cellulosic fibres (ACF), including dyed cotton, dyed cellulose, and indigo blue fibres (32.4 %, n = 22); (2) textile-derived microplastics, comprising PET–cotton (PET–COT), PET–cotton–polyurethane (PET–COT–PU), and cotton–polyurethane (COT–PU) composites (32.4 %, n = 22); and (3) petroleum-derived microplastics, including polyurethane (PU), polypropylene (PP), PVC, and other synthetic polymers (35.3 %, n = 24). This classification highlights the predominance of textile-related materials (64.7 % combined), while petroleum-derived polymers accounted for a slightly larger individual share (35.3 %). The most abundant individual polymers were modified cotton (ACF; 23.5 %, n = 16) and PET–COT (textile-derived; 23.5 %, n = 16), followed by PU (11.8 %, n = 8), PP (7.4 %, n = 5), PET–COT–PU (7.4 %, n = 5), and dyed cellulose (5.9 %, n = 4). Less common polymers (≤2.9 %, n ≤ 2) included indigo blue fibre (ACF), PVC, polyamide (PAP), acrylonitrile butadiene styrene (ABS), polyethylene (PE), polyethylene terephthalate (PET), polycarbonate-polyphenylene oxide (PC-PPO), polyethylene-co-polypropylene (PE-PP), polystyrene (PS), and polyethylene-vinyl acetate (PE-PEVA), all classified as petroleum-derived MP except for the ACF items (selected spectra in Figures S1-S7).

Polymer group composition varied across tissues (Figure S8 and S9). Gills (n = 7) were dominated by textile-derived microplastics (42.9 %) and petroleum-derived MP (42.9 %), with smaller contributions from ACF (14.3 %). The gastrointestinal tract (n = 22) showed the highest proportion of petroleum-derived MP (50.0 %), with ACF (27.3 %) and textile-derived MP (22.7 %) contributing comparably. In liver (n = 26), textile-derived MP was most abundant (38.5 %), followed by ACF (34.6 %) and petroleum-derived MP (26.9 %). Muscle tissue (n = 13) contained the highest proportion of ACF (46.2 %), with textile-derived MP and petroleum-derived MP each occurring at lower proportions (30.8 % and 23.1 %, respectively). These patterns indicate distinct uptake and translocation processes, with textile-derived materials broadly distributed across tissues, whereas petroleum-derived MP are more strongly represented in the GIT.

Shape-based patterns provided further insight into sources and pathways. Fibres (n = 205) were overwhelmingly dominant, typically blue (41 %), green (17 %), or transparent (12 %), and most were> 1 mm in length (42 %). Among Raman-confirmed fibres, the majority were PET–COT blends (23.5 %), modified cotton (23.5 %), or PU (11.8 %), reinforcing the dominance of textile-derived sources. Fragments (n = 27) were fewer and concentrated in the ≤ 100 µm and 100–500 µm ranges; Raman-confirmed fragments were petroleum-

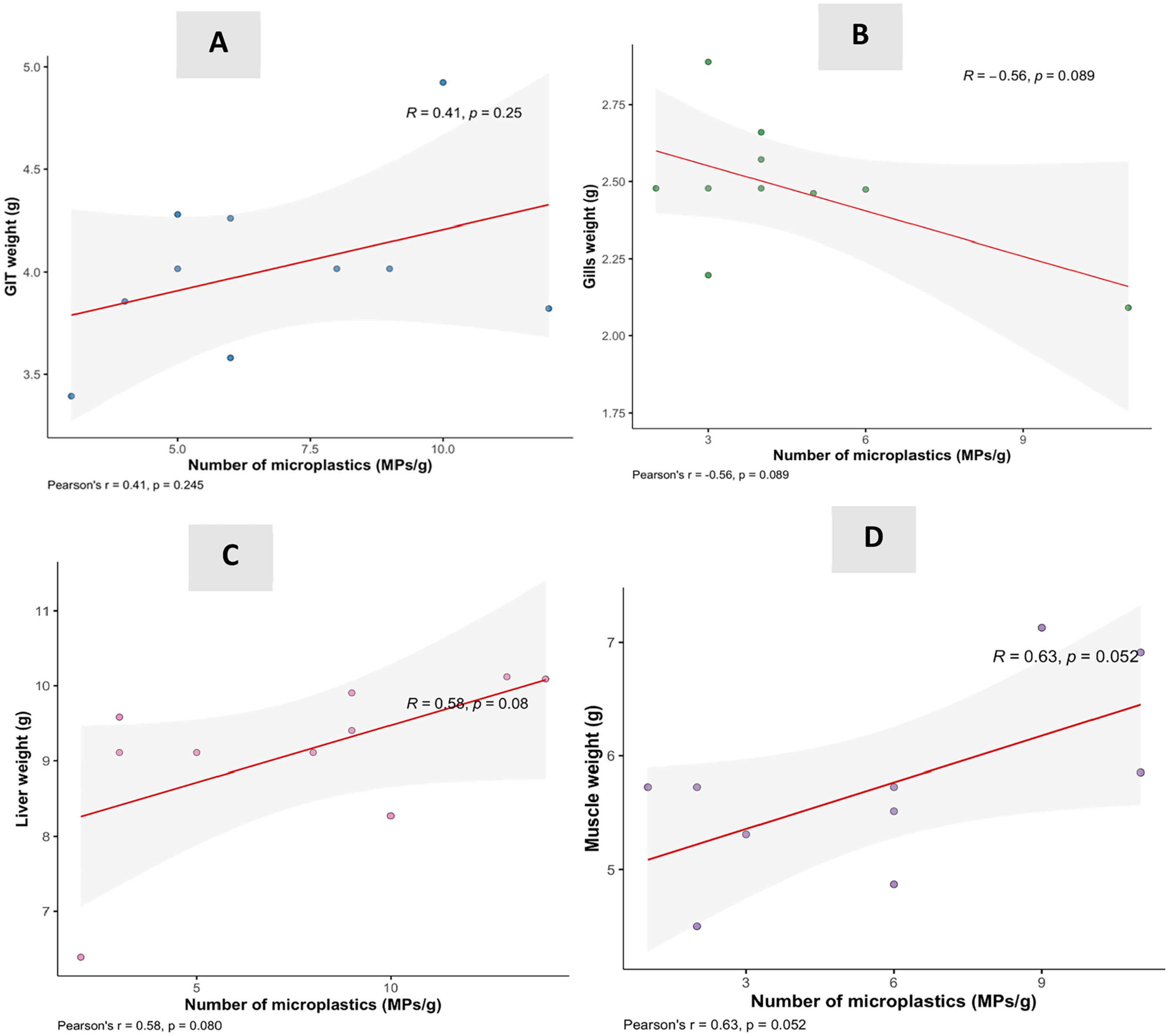

Fig. 3. Bivariate plots of the identified suspected microplastics per gram (MPs/g) (A) GIT weights, (B) Gill weights, (C) Liver weights, and (D) Muscle weights. The solid lines represent predicted associations, while the shaded areas are the 95% confidence intervals. R and *p* values are from Pearson correlation analysis.

derived (PET and ABS), consistent with coatings or rigid composites rather than packaging plastics. Sheets (n = 11) showed similar size distributions and were either PU (petroleum-derived) or modified cotton (ACF), indicating links to laminated or coated textile materials. Overall, cross-characterisation reveals a coherent pattern: blue, textile-associated fibres, both modified cellulosic and synthetic blends, dominate the microplastic profile, while smaller fragments and sheets are relatively scarce and primarily associated with petroleum-derived composites. These relationships clarify the predominance of textile inputs to the upper Tisza River and help distinguish them from packaging-related or PVC-based contamination pathways.

3.5. Estimated microplastic intake from burbot consumption

Human exposure estimates were calculated using EFSA weekly fish-consumption data [48] and body-weight assumptions [50], following the deterministic model of Matias *et al.* [47]. Based on the mean MP concentration in burbot dorsal muscle (0.96 ± 0.56 MPs/g), estimated intake varied markedly across age groups (Table 3). Infants consuming 15 g of fish per week were estimated to ingest approximately 14.4 MPs/week, equivalent to 2.88 MPs/kg bw/week. Toddlers showed a broader range of exposure (8.06–194.88 MPs/week; 0.67–16.24 MPs/kg bw/week), depending on weekly consumption. Older children and adolescents were estimated to ingest 14.78–206.98 MPs/week (0.64–8.96 MPs/kg bw/week) and 29.57–244.61 MPs/week (0.56–4.67 MPs/kg bw/week), respectively. For adults in the general European population, weekly exposure ranged from 32.26 to 320.54 MPs/week, normalised to 0.46–4.58 MPs/kg bw/week. Hungarian adults, who consume an average of 129 g of fish per week [49], had an estimated intake of 123.84 MPs/week, equivalent to 1.77 MPs/kg bw/week. On an annual scale, estimated intake ranged from 748.8 MPs/year in infants to 16,668 MPs/year in adults with high weekly consumption. When expressed relative to body weight, annual exposures ranged from 23.92 to 238.16 MPs/kg bw/year in adults and were proportionally higher in younger age groups due to lower body weights. These results demonstrate that both consumption habits and body-weight-normalised exposure strongly influence MP intake from freshwater fish.

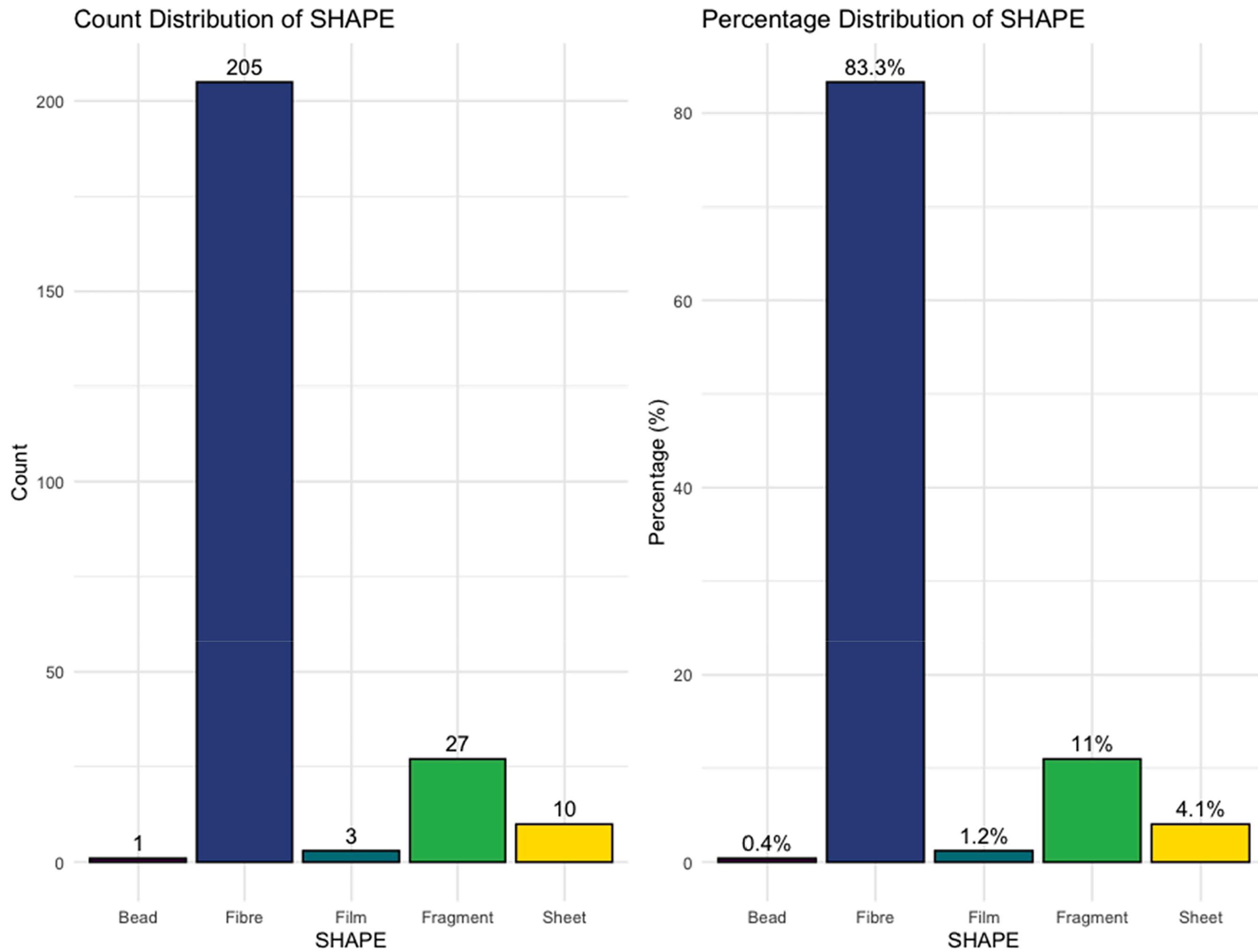

Fig. 4. Shape distribution (count and percentage) of suspected microplastics in fish samples.

4. Discussion

4.1. Abundance and distribution of microplastics

The widespread presence of MPs in aquatic environments has emerged as a global environmental concern, with mounting evidence documenting their retention across a broad spectrum of species, ecological settings, and feeding guilds [53]. While the majority of MP research has historically focused on marine ecosystems, freshwater systems, such as rivers, are increasingly recognised as critical conduits and reservoirs of plastic pollution [54]. In particular, the Hungarian reaches of the Tisza River, especially the upper boundary near Ukraine and the downstream sector bordering Serbia, have been flagged as hotspots of MP contamination. These patterns are primarily attributed to inadequate wastewater treatment infrastructure and ongoing cross-border pollution inputs [5,9,29]. Earlier studies have recorded significant levels of MPs in this river, with concentrations ranging from 4 to 63 particles/m^3 in surface waters and 528–8067 particles/kg in sediments. Bivalves, such as mussels, have been effectively used as sentinel organisms in these contexts [25]. Extending this line of inquiry, the current study introduces burbot, a benthic freshwater predator, as a novel bioindicator for assessing MP pollution in the upper Hungarian section of the Tisza River. This species is ecologically relevant due to its feeding habits and has commercial value as a fishery resource.

Across the 10 specimens analysed, the average MP load was 24.6 ± 7.46 particles per individual and 188.48 ± 58.44 particles/kg of body weight (equivalent to 0.19 ± 0.06 particles/g). As this work represents the first assessment of microplastic contamination in burbot from the Tisza River, it should be regarded as a baseline study. Consequently, the modest sample size reflects the exploratory nature of this initial investigation. These findings reinforce concerns over the growing MP burden in the Tisza River, particularly in upstream segments where rural Ukrainian communities frequently discharge untreated effluent into tributaries [5,9]. The region's steep catchment gradients and fast-flowing waters further facilitate the downstream mobilisation of MPs, amplifying contamination in Hungarian waters. Wastewater discharge remains a major contributor to riverine MP pollution, and its effects are increasingly evident in organisms that occupy these systems [23,55]. Given its benthic and predatory lifestyle, the burbot is susceptible to MP ingestion through both direct consumption of contaminated substrates and indirect trophic transfer from prey such as invertebrates and smaller fish [46,56]. These findings support the potential use of burbot as an indicator species for assessing microplastic contamination in freshwater systems, complementing existing evidence from mussels collected downstream [25] and other freshwater fish species [57,58]. Comparisons with published studies reveal that MP levels in burbot from the Tisza River fall within the range reported for many benthic and demersal fishes. For instance, similar elevated MP burdens have been documented in demersal carp from China's Han River (59.4 ± 45.5 particles/individual) [59] and South Africa's Vaal River (26.23 ± 12.57 particles/individual) [23]. In contrast, substantially lower concentrations (0.07–0.53 particles/individual) have been

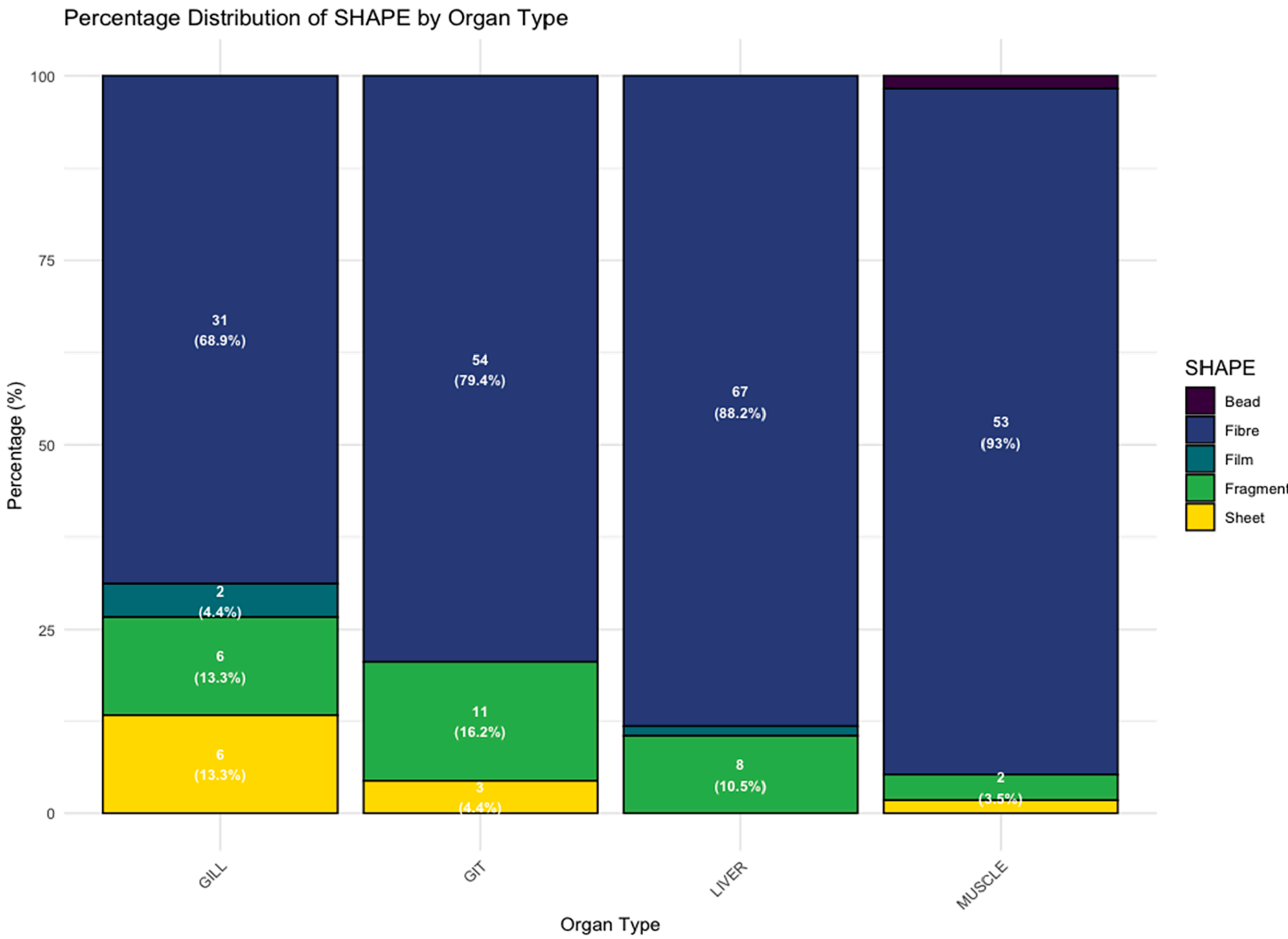

Fig. 5. Shape distribution (count and percentage) of suspected microplastics in different burbot tissues

observed in pelagic fish from coastal Kerala, India [60]. These contrasts likely reflect differences in habitat exposure and foraging behaviour [61–63]. However, it is essential to emphasise that interpretations drawn from the present dataset are constrained by the relatively small sample size ($n = 10$), which limits statistical power and may reduce the reliability of interspecific comparisons. Thus, the patterns described here should be viewed as indicative rather than definitive, emphasising the need for larger-scale sampling to validate species- and habitat-specific trends in MP accumulation.

4.2. Tissue-specific distribution of microplastics

Our findings demonstrate apparent tissue-specific differences in microplastic retention in burbot, consistent with organ-level variation in exposure pathways and physiological function. Based on median concentrations, the GIT (1.54 MPs g^{-1}) and gills (1.53 MPs g^{-1}) harboured the highest MP burdens, while muscle (1.07 MPs g^{-1}) and especially liver (0.89 MPs g^{-1}) contained markedly fewer particles. This pattern was supported by nonparametric analyses, which revealed significantly lower MP concentrations in the liver than in the GIT and gills. In contrast, muscle showed intermediate values without significant pairwise differences. These findings reinforce the role of the GIT as the primary ingestion route and the gills as a substantial site of particle interception during respiration [32,64].

The presence of MPs in liver and muscle tissues, albeit at low levels, suggests that some internal translocation may occur following entry through the GIT or gills; however, this interpretation remains tentative in the absence of histological confirmation or size-resolved uptake data. While systemic pathways such as circulatory transport, endocytosis, or persorption have been proposed in vertebrates [42,46,62], the current study cannot verify whether these mechanisms operate in burbot. Similar detection of MPs in internal organs has been reported in several fish species. However, patterns vary widely and appear strongly influenced by species-specific anatomy, metabolic activity, and exposure conditions [32,60,64]. In the present study, MP concentrations were consistently lower in internal edible tissues than in non-edible filtration or ingestion-related tissues, consistent with broader literature trends. Nonetheless, the detection of MPs in liver and muscle, organs relevant to human consumption, emphasises the need for targeted studies using imaging, histopathology, and particle-size analyses to elucidate the actual translocation pathways and assess their implications for food safety.

The variability in MP retention among tissues is likely influenced by both particle-specific characteristics (e.g., size, shape, polymer type) and tissue-level physiological factors such as vascularisation, epithelial permeability, and organ-specific uptake dynamics [32,65]. Although correlation analysis did not reveal any statistically significant associations between tissue mass and MP abundance ($p \geq 0.05$), the observed correlation coefficients suggest possible, but unconfirmed, tendencies. For example, the moderate positive coefficients for liver ($r = 0.58$) and muscle ($r = 0.63$), and the moderate negative coefficient for gills ($r = -0.56$), may indicate patterns worth investigating in larger datasets. However, these should not be interpreted as real or biologically meaningful relationships, given the lack of statistical support and the limited sample size. Instead, they highlight potential directions for future research to clarify whether tissue morphology or size plays a role in MP

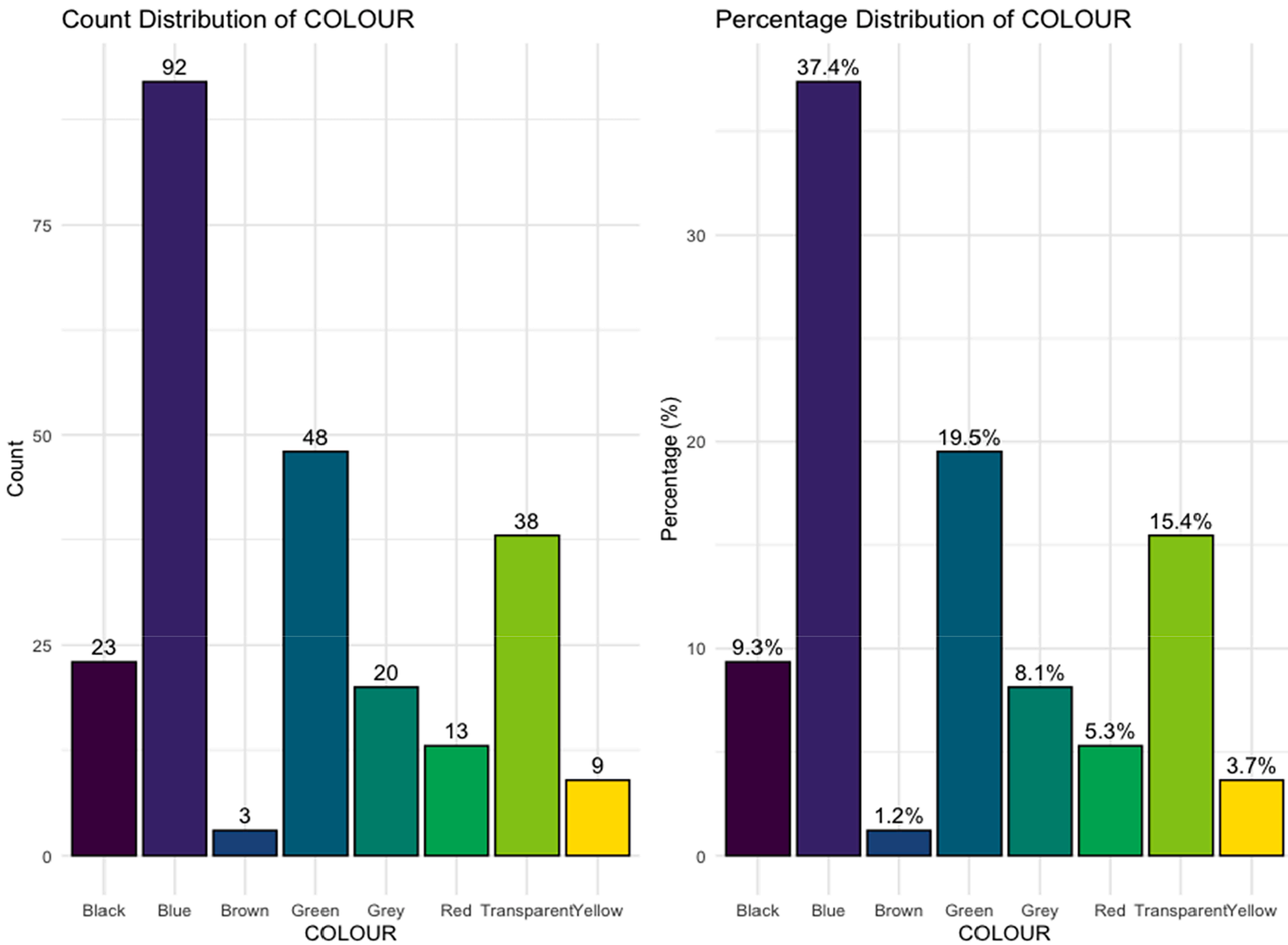

Fig. 6. Colour distribution (count and percentage) of suspected microplastics in fish.

accumulation. Previous studies have reported mixed outcomes: some species show no relationship between organ mass and MP load [54], while others show significant associations [32,59], reflecting the complexity and species-specific nature of MP dynamics.

4.3. *Morphological characteristics of microplastics in burbot tissues*

The physical characteristics of MPs, particularly their shape, size, and colour, provide valuable information about their likely sources, environmental pathways, and interactions with aquatic organisms [66]. In natural water bodies, fish may ingest MPs that visually or texturally resemble their typical prey, highlighting the pervasive bioavailability of these pollutants [56]. In this study, five distinct MP morphologies were observed in burbot individuals: fibres (83.3 %, $n = 205$), fragments (11 %, $n = 27$), sheets (4.1 %, $n = 10$), films (1.2 %, $n = 3$), and beads (0.4 %, $n = 1$). The predominance of fibrous particles is consistent with global freshwater and marine datasets, which show that fibres released from household textiles, fishing gear, and wastewater effluent are standard [34,64]. In particular, domestic laundry has been recognised as a key contributor of fibrous MPs to aquatic systems. Studies show that a single wash of synthetic garments can shed up to 1900 fibres [67,68], many of which escape wastewater treatment and are discharged into surface waters via drainage canals or effluent streams [21,23,69].

Within the Tisza River basin, especially in its upper reaches near the Ukrainian border, microfibres account for 84–98 % of MPs found in both surface waters and sediments, a trend attributed to poor wastewater infrastructure in upstream regions [5,9]. Fibre-rich effluents, comprising up to 90 % of MPs in untreated wastewater, present a substantial and persistent source of contamination [70,71]. The elongated and flexible nature of these fibres may increase their likelihood of being ingested by burbot, a demersal predator that includes invertebrates and smaller fish in its diet [34]. The high prevalence of fibres in edible tissues, 92.98 % in muscle and 88.2 % in the liver, also suggests that fibres may more easily translocate from the GIT or gills into internal organs [72], raising significant concerns about MP retention and potential human exposure through fish consumption. Fragments, the second most commonly encountered morphology (11 %), were particularly abundant in the GIT (16.18 %), likely ingested along with prey or sediment particles containing degraded plastic residues. The relative scarcity of beads and films may reflect their lower environmental occurrence or limited ingestion due to particle shape or behaviour. While fibre dominance is a consistent trend in freshwater MP studies [23,45,46,64], some marine environments report higher proportions of fragments, suggesting possible ecosystem-level differences in MP composition and exposure [42,73,74].

MP colour can also provide valuable clues about the origin of particles. In aquatic systems, coloured fibres, often blue or green, are typically associated with dyed textiles. In contrast, transparent and white particles are associated with packaging materials, while black particles are linked to sources such as tire wear or road runoff [23]. In the present study, blue (37.4 %), green (19.5 %), and transparent (15.4 %) MPs were the most frequently observed colours, consistent with patterns reported in other freshwater and marine species [38,46,75]. Blue fibres, commonly derived from fabrics such as denim, are especially prevalent in the Tisza River, likely stemming from upstream wastewater discharges [5,76]. These coloured particles may be mistaken

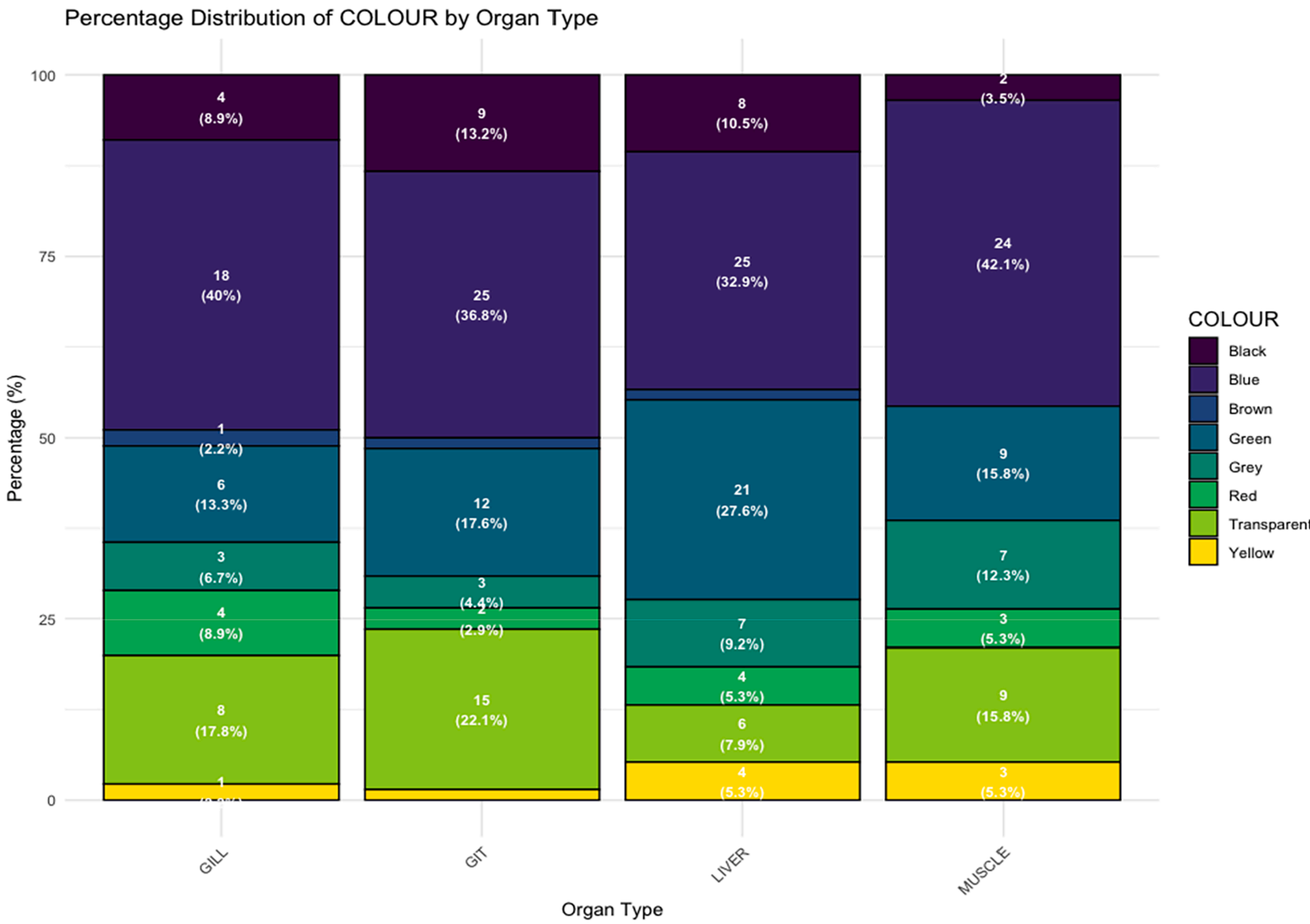

Fig. 7. Colour distribution (count and percentage) of suspected microplastics in different burbot tissues.

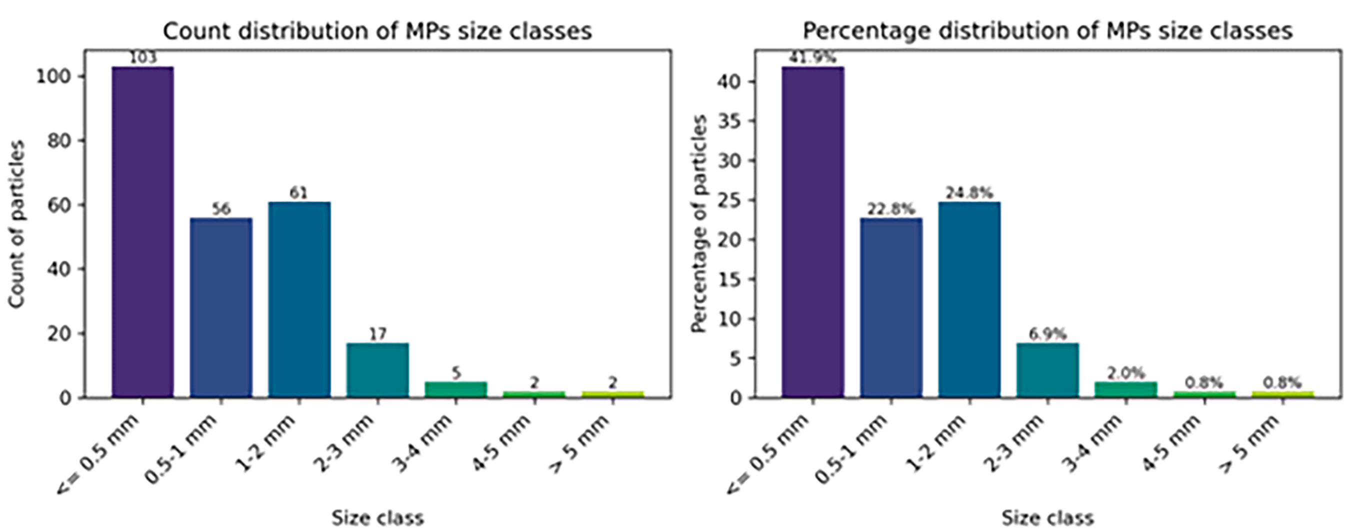

Fig. 8. Size distribution (count and percentage) of suspected microplastics in burbot tissues.

for natural prey, increasing the likelihood of ingestion by fish [77,78]. Transparent particles may mimic plankton or suspended organic matter, further enhancing their uptake [79]. While environmental degradation can alter particle colour over time [80], blue hues appear particularly attractive to fish species [81]. Moreover, coloured MPs pose additional risks due to the leaching of dyes and chemical additives upon ingestion and subsequent metabolism by aquatic organisms [23].

In terms of size, MPs smaller than 500 μm accounted for the most significant proportion (41.9 %) of all particles recovered, with their presence particularly high in the gills (46.7 %), liver (42.1 %), and muscle (40.4 %) compared to the GIT. Particle size plays a central role in determining environmental fate, biological uptake, and toxicological potential [23]. Smaller MPs are more mobile, can travel farther in aquatic systems, and are more bioavailable to a broader range of

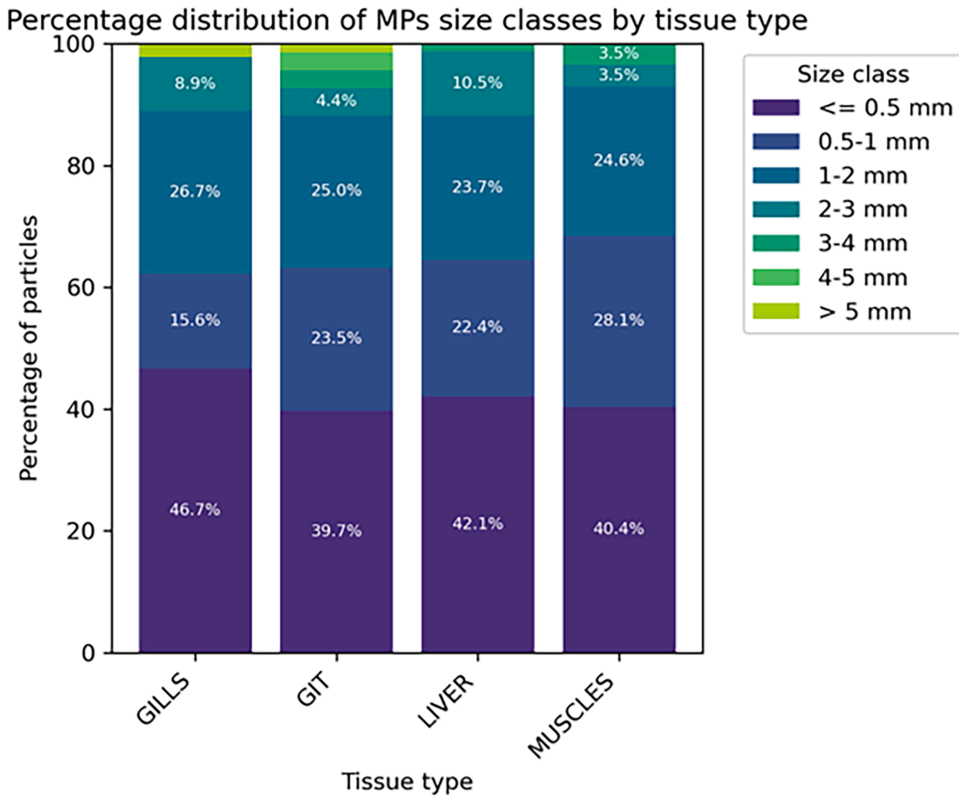

Fig. 9. Size distribution (count and percentage) of suspected microplastics in different burbot tissues.

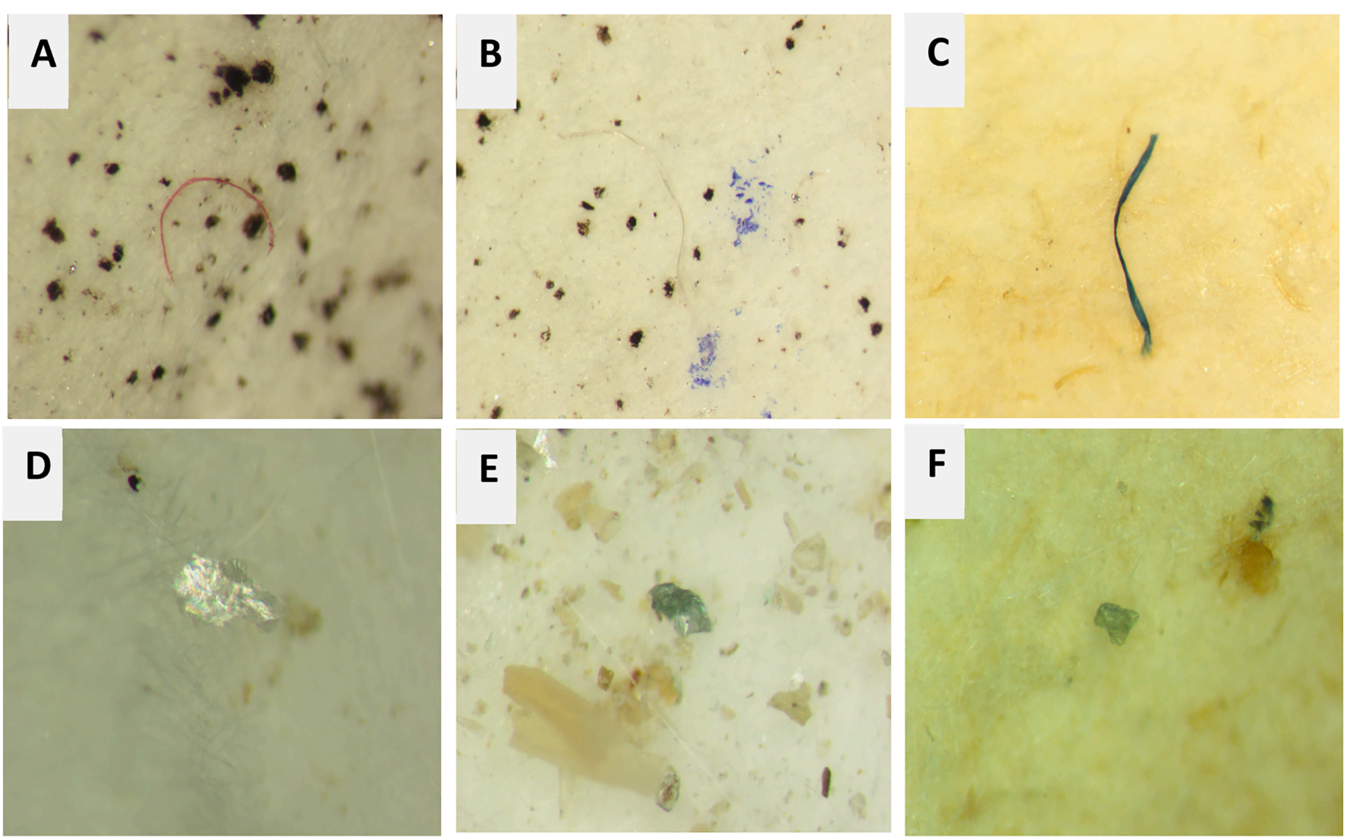

Fig. 10. Stereomicroscope images of identified suspected microplastics in various burbot tissues: (A) Red fibre, (B) Transparent fibre, (C) Blue fibre, (D) Transparent sheet, (E) Green sheet, and (F) Green fragment.

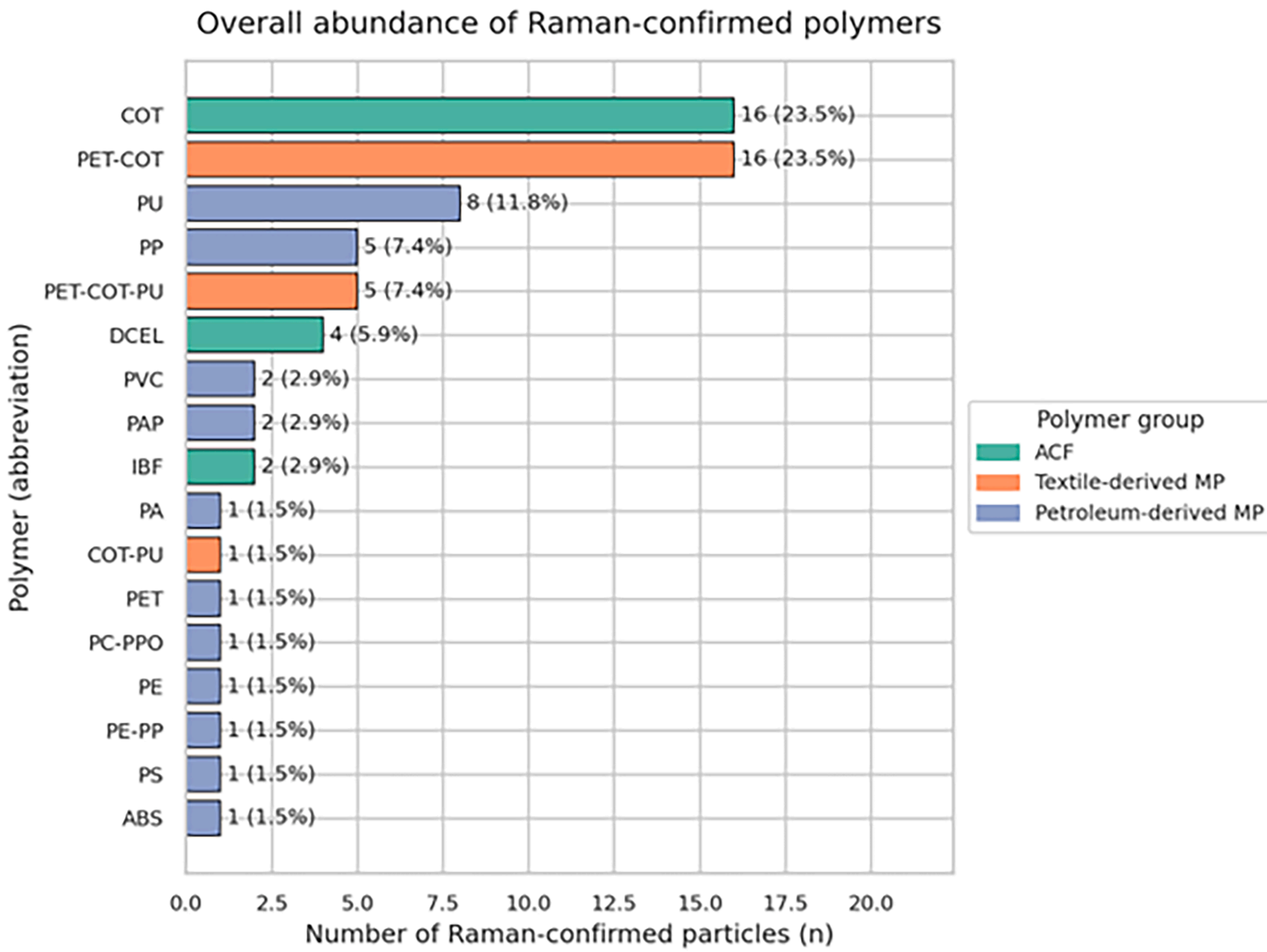

Fig. 11. Percentage distribution of chemical properties of suspected microplastics identified in the burbot. Acrylonitrile Butadiene Styrene (ABS), Cotton (COT), Cotton-Polyurethane (COT-PU), Dyed Cellulose (DCEL), Indigo Blue-Fibre (IBF), Polyamide (PA), Polycarbonate and Polyphenylene Oxide (PC-PPO), Polyethylene (PE), Polyethylene Terephthalate (PET), Polyethylene Terephthalate-Cotton (PET-COT), Polyethylene Terephthalate-Cotton-Polyurethane (PET-COT-PU), Polyethylene-Co-Polyethylene Vinyl Acetate (PE-PEVA/PAP), Polyethylene-Co-Polypropylene (PE-PP), Polypropylene (PP), Polystyrene (PS), Polyurethane (PU), and Polyvinyl Chloride (PVC).

Table 3
Estimated annual human microplastic (MP) intake from burbot consumption across age groups based on weekly intake and EFSA body weight assumptions.

Age group	Mean Fish Cons. (g/week)	BW (kg)	Estimated MP intake (part/week)	Estimated MP intake (part/kg bw/week)	Estimated MP intake (Part/year)	Estimated MP intake (Part/kg bw/year)
Infants (0–12 months)	15	5	14.40	2.88	748.80	149.76
Toddlers (1–3 years)	8.4–203	12	8.06–194.88	0.67–16.24	419.12 – 10,133.76	34.84 – 844.48
Other children (36 mo–10 y)	15.4–215.6	23.1	14.78–206.98	0.64–8.96	768.56 – 10,762.96	33.28 – 465.92
Adolescents (10–18 y)	30.8–254.8	52.4	29.57–244.61	0.56–4.67	1537.64 – 12,719.72	29.12 – 242.84
Adults (General European population)	33.6–333.9	70	32.26–320.54	0.46–4.58	1677.52 – 16,668.08	23.92 – 238.16
Adults (Hungarian population)	129	70	123.84	1.77	6439.68	92.04

Cons.: Consumption; BW: Body Weight; Mo: Month; Y: Year; Part.: Particles.

organisms [82–84]. Their compact size and high surface-area-to-volume ratio make them more likely to bypass intestinal barriers, become temporarily retained in tissues, and persist in biological systems [64,85]. The predominance of small MPs in internal organs suggests enhanced translocation potential, supported by findings from similar studies in other freshwater species [46,64,82,86]. Their abundance may also result from the ongoing environmental degradation of larger plastics and the increasing prevalence of microplastics in natural systems [21,87]. Smaller individuals, such as juvenile burbot, may be particularly vulnerable due to their feeding strategies and physiological limitations [59]. Significantly, small MPs are associated with elevated toxicological risk, as they can penetrate cells and tissues and serve as carriers for other hazardous substances, including heavy metals, pathogens, and persistent organic pollutants [22,88,89]. Their potential to cause oxidative stress, inflammatory responses, and systemic toxicity underscores the importance of investigating their role in both ecological and human health contexts. Together, these findings highlight the importance of ongoing research into how MP morphology affects ingestion, tissue distribution, and toxicity, particularly in commercially valuable fish species and regions experiencing increased pollution pressure.

4.4. Polymer Composition and Tissue-Specific Distribution of Identified Particles

Raman spectroscopy confirmed the polymer identity of 68 microplastic particles, representing 17 distinct polymers and copolymers. Analysis of the spectra revealed that all cellulosic fibres exhibited clear anthropogenic modification (dyeing, indigo pigmentation, coating, or blending with synthetic polymers), confirming that none originated from unmodified natural sources. Consequently, textile-related materials, combining ACF and textile-derived synthetic blends, accounted for 64.7 % of all identified MPs. The most prevalent individual polymers were modified cotton (ACF; 23.5 %) and PET–cotton blends (textile-derived MP; 23.5 %), followed by polyurethane (11.8 %), polypropylene (7.4 %), and PET–cotton–polyurethane composites (7.4 %). Conventional packaging plastics such as PE, PVC, and PS were observed only sporadically, indicating that rigid packaging debris makes a minor contribution relative to fibre-rich textile sources. This polymeric signature is consistent with increasing evidence that wastewater effluent, domestic laundry discharge, and urban runoff are primary sources of MP inputs to the upper section of the Hungarian Tisza River [90]. The cross-characterisation of polymer, colour, and shape further supports a predominantly textile-originated contamination pathway. Fibres, by far the dominant shape class, exhibited intense colour–polymer associations across tissues. Blue fibres, comprising ~41 % of all fibres, were primarily PET–COT blends and dyed/modified cotton, while green and transparent fibres were frequently composed of polyurethane or dyed cellulose derivatives. These polymers are hallmark components of everyday clothing, especially denim, athletic wear, and coated fabrics. The high proportion of polyurethane-containing fibres and composites suggests substantial contributions from laminated or performance textiles, which are known to shed heavily during washing and resist removal in wastewater treatment [91–94]. The frequent indigo pigmentation observed in many PU- and PET–cotton fibres (Fig. 12) further implicates denim as a significant source, consistent with the findings that up to 51 % of sedimentary MPs in this region consisted of indigo-dyed denim microfibres released during laundering [95]. Numerous studies report that laundering denim garments can release between 0.64–1.5 million microfibres per kilogram of fabric, many of which evade filtration systems and enter natural waterways [25,96,97].

In contrast, fragments and sheets were rare and exhibited distinct profiles. Fragments were small (≤500 µm), mostly green or transparent, and, when confirmed by Raman, composed of petroleum-derived PET or acrylonitrile–butadiene rather than the PE, PE–VA, or PVC typically associated with packaging. Sheets were similarly scarce and composed mainly of polyurethane or modified cotton, suggesting origins in textile coatings or laminated materials rather than conventional plastic films. These patterns collectively indicate that rigid, packaging-derived MPs play a comparatively minor role in the microplastic burden carried by burbot in this study. The distribution of polymer groups across tissues provides further insights into exposure pathways. Gills were co-dominated by textile-derived MP and petroleum-derived MP (each 42.9 %), reflecting direct filtration of suspended textile fibres from the water column. The gastrointestinal tract exhibited the highest proportion of petroleum-derived MP (50.0 %), consistent with ingestion of a broader range of particle types (supplementary figure X), including those associated with sediments or prey. Liver and muscle tissues showed progressively higher contributions from ACF and textile-derived blends (liver: 38.5 % textile-derived + 34.6 % ACF; muscle: 30.8 % textile-derived + 46.2 % ACF), suggesting selective translocation or retention of flexible, fibre-like particles across epithelial barriers [72]. The pervasive dominance of fibres across all tissues highlights the enhanced retention and bioadhesion potential of elongated particles, a trend increasingly observed in wastewater-influenced freshwater environments [46,64,98].

Overall, these findings reinforce growing recognition that microfibres, not fragments, represent the primary form of polymeric exposure for benthic fish inhabiting systems with limited wastewater treatment [98]. The close match between the polymer profiles of burbot and previously studied mussels [25] from the same river further indicates cross-trophic persistence of textile-derived MPs within the aquatic food web. Ecologically, the textile-centric profile suggests a concentrated

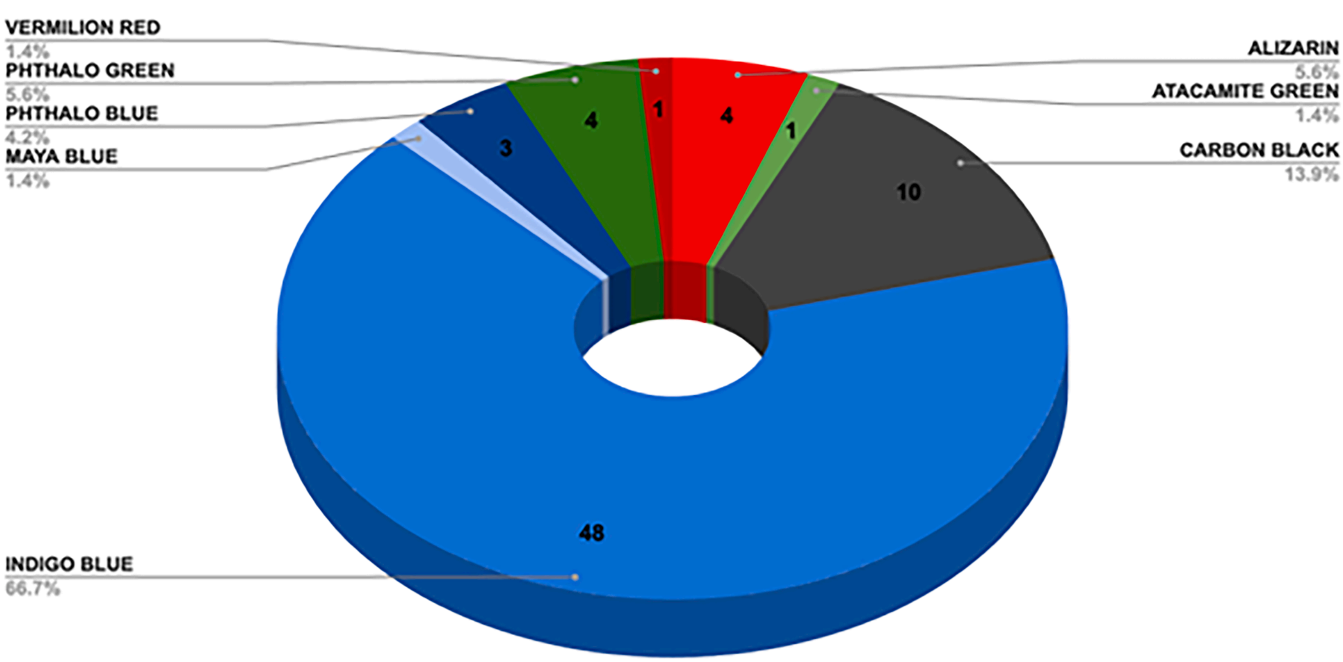

Fig. 12. Distribution of identified pigments in microplastics polymer samples. Composite polymer materials were excluded from the analysis, and only pigment components were considered.

contamination pathway driven by laundry-derived fibres and coated fabrics. PET–cotton and PU composites, in particular, are of concern because they combine durability with chemical complexity; they can carry dyes, PFAS, plasticisers, and other additives known to elicit oxidative, endocrine, and immunotoxic effects [99]. Their presence in edible muscle tissues, although limited in number, signals a direct and under-recognised exposure route for consumers of freshwater fish in regions affected by untreated or partially treated effluent.

In summary, burbot from the Tisza River accumulate a chemically distinctive, textile-dominated microplastic assemblage that reflects the pervasive influence of domestic laundering and urban wastewater inputs. The integrated analysis of polymer identity, colour, size, and shape provides a strong basis for source discrimination. It demonstrates that fibre-dominated pollution, rather than conventional plastic debris, defines the microplastic landscape of this river. These findings emphasise the urgent need for improved textile waste management, microfibre capture technologies, and enhanced regional wastewater infrastructure to mitigate the release and biological uptake of microfibres in Central European freshwater ecosystems.

4.5. Implications for freshwater ecosystems and human health

The pervasive presence of MPs in various tissues of burbot, including gills, GIT, liver, and edible muscle, raises substantial ecological and public health concerns stemming from their direct toxicity to aquatic organisms and the potential for dietary exposure in humans. MPs enter fish through multiple pathways, including ingestion of contaminated prey, gill retention during filtration, and dermal uptake. Once internalised, their dynamics involve excretion via faeces, retention in the GIT, or translocation to organs, thereby posing risks to fish health and ecosystem stability [23,46,67].

In freshwater and marine ecosystems, MPs exert detrimental effects on fish by obstructing and damaging intestinal tissues, reducing feeding efficiency, and inducing starvation, as fish may mistake MPs for natural prey, ultimately lowering their intake of real food sources [100]. These particles also trigger physiological disruptions, including metabolic imbalances, oxidative stress, immune dysfunction, inflammation, gut microbiota dysbiosis, genotoxicity, and, in severe cases, mortality, with both controlled exposure studies and environmentally relevant assessments demonstrating size- and dose-dependent effects [101–104]. Importantly, similar patterns are now reported in wild and farmed fish, where MP ingestion is associated with oxidative stress, neurotoxicity, and histopathological alterations in key organs such as gills, liver, and intestine, as well as altered antioxidant responses and acetylcholinesterase activity [35,46,105–108]. Behavioural impairments, such as reduced swimming ability and coordination, have also been reported in carp exposed to MPs, with consequences for predator avoidance and survival [22]. Together, these findings indicate that the sublethal effects observed under controlled exposure conditions are also detectable in free-living and aquaculture populations, reinforcing concerns that increasing MP burdens can compromise overall fish health status and, by extension, food-web and human health. Furthermore, the ecological ramifications extend beyond individual organisms, as MPs disrupt primary producers, such as algae and aquatic plants, by inhibiting photosynthesis and growth, while altering benthic invertebrate communities through digestive damage and reduced reproductive success, ultimately distorting predator-prey dynamics and threatening biodiversity [1,109].

MP toxicity represents a multifaceted threat, arising not only from the particles themselves but also from the diverse contaminants they transport, including heavy metals, per- and polyfluoroalkyl substances (PFAS) compounds, and antibiotic resistance genes, which together amplify its environmental and health risks [110–112]. These concerns are particularly relevant for populations with high freshwater-fish consumption, given the cultural and culinary value of species such as burbot across parts of Europe, including Belgium, Poland, Finland, and Austria [18,113,114]. However, EUMOFA's [115] retail-consumption data reveal that species such as salmon, trout, carp, and hake comprise the bulk of fish consumed in Europe. At the same time, burbot contributes only marginally to total intake. Because MP burdens differ substantially among species owing to variations in physiology, trophic ecology, and habitat exposure, the concentrations measured in burbot in this study should be interpreted as a proxy for potential exposure rather than a direct representation of overall dietary MP intake in Hungary or Europe. In this study, human exposure estimates were calculated using the updated EFSA weekly fish consumption data [48] and body-weight assumptions [50], following the deterministic model of Matias et al. [47]. Based on the mean MP concentration quantified in burbot muscle (0.96 ± 0.56 MPs/g), estimated intake varied substantially across age groups. Annual exposure ranged from approximately 749 MPs/year in infants to over 16,600 MPs/year in adults with high weekly consumption, while body-weight-normalised exposures were markedly higher in younger individuals. For Hungarian adults consuming an average of 129 g of fish per week [49], the estimated intake was 6439 MPs/year (92 MPs/kg bw/year), emphasising the role of consumption patterns in shaping exposure risks. Furthermore, food preparation and consumption practices can further influence contaminant loads and thus may either mitigate or exacerbate actual exposure relative to raw-tissue estimates [47].

Within this context, the exposure estimates derived from burbot muscle highlight several important patterns. First, although absolute MP intake from burbot remains lower than some global benchmarks, such as the widely cited ~55,000 MPs/year from seafood [116], our data fall above several baseline European estimates (e.g., 16 MPs/week or 842 MPs/year for Portuguese adults) [46], and therefore underline the potential significance of freshwater species as exposure pathways in polluted systems. Second, normalising intake to body weight reveals that younger age groups, particularly infants and toddlers, experience disproportionately higher exposures relative to adults, possibly due to their lower body weight. This observation aligns with EFSA's recognition of heightened vulnerability in early life. It corresponds with recent findings by Matias et al. [47], who demonstrated several-fold greater MP intake in toddlers than in adults.

While the estimated exposures reported here are comparable to those documented in other freshwater systems (e.g., ~299 MPs per 300 g of fish in the Persian Gulf [65]), they remain far below the levels observed in regions where whole-fish consumption is typical, such as West Africa, where annual intakes may exceed 178,000 MPs/year [117]. Importantly, the present results also suggest that consumption of organs such as burbot liver, when culturally relevant, could modestly elevate overall MP exposure. Overall, these findings reinforce the need for broader, multi-species monitoring programmes that reflect actual consumption patterns and capture species-specific variability in MP retention. Future work should prioritise commonly consumed species, such as carp, and incorporate other benthic taxa to broaden the representation of sediment-associated MP transfer in lotic ecosystems. This approach will refine human health risk assessments and enhance our understanding of MP exposure across diverse dietary and ecological contexts.

Human exposure to MPs extends beyond diet, with detections in blood, lungs, liver, placenta, milk, and other tissues, indicating widespread contamination through drinking water, inhalation, and other dietary sources [118–120]. The retention of MPs in human tissues can trigger cardiovascular, digestive, respiratory, and genotoxic effects, as well as endocrine disruption [121–123], which are modulated by particle characteristics (size, shape, surface charge) and their capacity to sorb contaminants [124–126]. The presence of MPs in human stool confirms their passage through the digestive system, yet the long-term health implications remain poorly understood, necessitating urgent research [46,127]. Vulnerable populations, such as infants and children, face heightened risks due to developing physiological systems, emphasising the need for targeted dietary guidelines.

These findings highlight the critical role of freshwater fish as a conduit for MP exposure, particularly in communities with poor plastic waste management and that rely on species like burbot, and shed more light on the broader ecological disruptions caused by MPs. To mitigate these risks, comprehensive monitoring of MP contamination in aquatic ecosystems, establishment of regulatory thresholds for MPs in food, and reduction of plastic pollution through improved waste management and sustainable alternatives are imperative. This study contributes to the growing body of evidence that MPs pose a pressing environmental and public health challenge, underscoring the need for interdisciplinary action to protect freshwater ecosystems and human well-being.

Limitations of the Study

The authors acknowledge several limitations that should be considered when interpreting the outcomes of this research:

- **Sample Size Constraints**
  This study examined ten burbot individuals, a number below internationally recommended thresholds for standardized monitoring (e.g., MSFD, OSPAR). This constraint reflects practical challenges associated with burbot ecology, including seasonal behaviour, low catchability, and naturally low abundance at the study site. As a result, statistical inference is limited, and the findings should be interpreted as exploratory baseline data rather than population-level estimates.
- **Environmental Context (absence of water and sediment sample-based analysis)**
  This study did not include concurrent water or sediment sampling at the fish collection site. Our design focused on organism-level contamination and tissue-specific accumulation rather than short-term environmental concentrations. Although burbot provides an integrated, long-term exposure signal, the absence of ecological matrices limits direct comparison between fish-derived microplastics and those present in the surrounding habitat during sampling. Future work combining biological and environmental sampling would better resolve exposure pathways. Nevertheless, comparisons with existing water and sediment data from the Tisza River remain informative given the availability of relevant published studies.
- **Restricted Spatial Coverage**
  Sampling was conducted exclusively at a single location (Tiszabecs) within the upper Tisza River. Microplastic contamination can vary considerably across river gradients due to differences in hydrodynamics, anthropogenic inputs, and sediment characteristics. Therefore, the results do not capture potential spatial heterogeneity across the broader Tisza River basin.
- **Lack of Temporal Resolution**
  All specimens were collected during a single sampling campaign. Microplastic abundance in freshwater environments often exhibits substantial temporal variability driven by seasonal flow patterns, precipitation events, and episodic pollution. The absence of multi-season sampling limits the temporal representativeness of the findings.

5. Conclusion

This study provides the first comprehensive assessment of microplastic pollution in fish from Hungary and the first investigation of microplastics in burbot across the Carpathian Basin. Our results revealed ubiquitous contamination in burbot from the Hungarian section of the Tisza River, with a mean intensity of 24.6 ± 7.46 particles per fish. Detected particles were predominantly small (<500 μm), fibre-shaped, and blue, with the highest concentrations in gills (1.88 ± 1.28 MPs/g) and GIT (1.68 ± 0.69 MPs/g), and lower levels in liver (0.81 ± 0.41 MPs/g) and dorsal muscle (0.96 ± 0.56 MPs/g). The chemical analysis shows a preponderance of indigo-dyed PET, cotton, and cellulose-based polymers in the analysed samples. The findings of this study confirm the burbot's role as a sentinel species for monitoring riverine pollution and raising concerns about human exposure through consumption, with estimated weekly MP intake ranging from 38.4 particles for infants to 123.6 particles per capita in Hungary. The prevalence of textile-derived MP pollution, likely stemming from inadequate upstream waste management and wastewater treatment, establishes a critical baseline for Central European freshwater systems, advocating for robust waste management policies, enhanced wastewater treatment, and further research into MP retention, polymer-specific toxicity, and regional mitigation strategies to protect aquatic ecosystems and public health, while affirming burbot's significance in ongoing environmental monitoring and conservation efforts within the Tisza River basin.

Environmental Implication

Microplastic pollution in fish is a significant and pressing issue today.

The prevalence of textile-derived microplastic pollution, likely stemming from inadequate upstream waste management and wastewater treatment, establishes a critical baseline for freshwater systems in Central Europe.

Advocating for robust waste management policies, enhanced wastewater treatment, and further research into MP bioaccumulation, polymer-specific toxicity, and regional mitigation strategies to protect aquatic ecosystems and public health.

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CRediT authorship contribution statement

Nelson Ike Ossai: Writing – review & editing, Validation. **László Antal:** Writing – review & editing, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Dóra Somogyi:** Writing – review & editing, Investigation, Formal analysis. **Angéla Pásztor:** Writing – review & editing, Validation, Investigation. **Ifeanyi Emmanuel Uzochukwu:** Writing – original draft, Visualization, Software, Investigation, Data curation, Conceptualization. **László Nagy:** Writing – review & editing, Validation, Resources. **István Csarnovics:** Writing – review & editing, Validation, Investigation. **Krisztián Nyeste:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Vesela Yancheva:** Writing – review & editing, Validation, Investigation.

Declaration of Generative AI and AI-assisted technologies in the writing process

The author(s) utilised the artificial intelligence tools ChatGPT and Perplexity to correct grammar and enhance the manuscript's readability during its preparation. After employing this tool/service, the author(s) critically reviewed and edited the content as necessary and assume complete responsibility for the publication's accuracy and integrity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2026.141048.

Data availability

Data will be made available on request.

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