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Falling from the ceiling: first evidence of microplastic and microfibre pollution in cave dripping waters

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Research paper

Falling from the ceiling: first evidence of microplastic and microfibre pollution in cave dripping waters

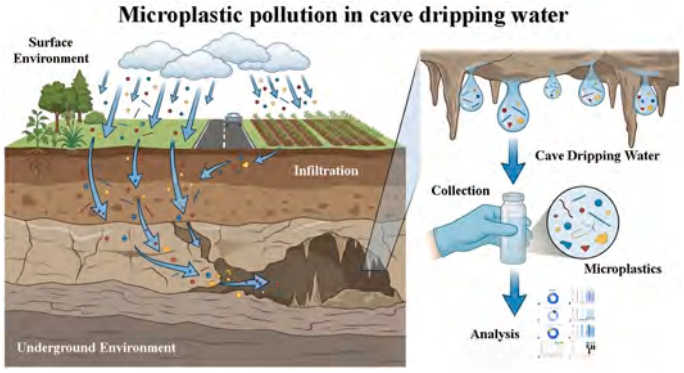
Valentina Balestra [a,b,c,*], Raffaella Mossotti [d], Luca Zini [e], Rossana Bellopede [b]

[a] Department of Agricultural, Forest & Food Sciences, University of Turin, Italy
[b] Department of Environment, Land and Infrastructure Engineering, Politecnico di Torino, Italy
[c] Biologia Sotterranea Piemonte – Gruppo di Ricerca c/o Bossea Cave, Italy
[d] Institute of Intelligent Industrial Technologies and Systems for Advanced Manufacturing, National Research Council, Italy
[e] Department of Mathematics, Informatics and Geosciences, University of Trieste, Italy

HIGHLIGHTS

- Cave dripping waters are polluted by microplastics and microfibres.
- Small size cellulosic microfibres and synthetic fragments dominated the water samples.
- Microplastics were especially PET and PU.
- Human activities and land pollution impact indirectly karst systems.
- Monitoring subterranean environments is fundamental for karst system and water conservation.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastic (MP) pollution is a global environmental concern. Recent works highlighted the need to consider even natural and regenerated microfibres (MFs). Despite their ecological and hydrological importance, karst systems remain understudied. Being open systems, karst underground environments are particularly vulnerable to contamination through the connections with surface habitats. For the first time, this preliminary research investigated the presence of MPs and other anthropogenic microparticles (AMPs), such as MFs, in cave dripping waters, highlighting the indirect impact of surface human activity. AMPs were counted and characterized via microscopy and spectroscopy. The 80.5% of the analyzed AMPs were cellulosic and 81.6% fibres. MPs ranged from 0 to 23 MP/L, confirming the presence of polyethylene terephthalate (PET) and polyurethane (PU), and were all fragments, except for one PET fibre. Microparticles < 1 mm were the most abundant (89.3%), of which the most had size between 0.49 and 0.10 mm (51.5%). Pollution sources in cave dripping waters could be linked to landfills in sinkholes, garbage, nearby roads, railways, and human activities. These results highlight MPs and other AMP pollution exists in cave dripping waters, impacting water resources, habitats and species. More

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* Corresponding author. Department of Agricultural, Forest & Food Sciences, University of Turin, Italy.
E-mail address: valentina.balestra@unito.it (V. Balestra).

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comprehensive and multidisciplinary monitoring are needed, taking into account surface and subterranean connections in karst areas, aiming at conservation purposes. Promoting collaborations with show caves and cavers can be instrumental in pilot studies, and acquiring new and valuable data.

1. Introduction

Microplastics (MPs) were first defined by Thompson et al. (2004) as plastic particles smaller than 5 mm, originating from the fragmentation of larger plastic or intentionally manufactured at small size. Subsequently, this definition has been adopted and refined by scientific and environmental organizations, typically defining MPs as synthetic particles ranging from 5 mm to 1 μm (e.g. Frias and Nash, 2019; International Organization for Standardization and European Committee for Stanton et al., 2019). Recently, the term "big MPs" (5–1 mm) was introduced (International Organization for Standardization and European Committee for Standardization, 2020. However, the MP definition remains under debate. The recent definition in ISO 4484-2 (International Organization for Standardization, 2023) described MP as "material consisting of a solid polymer containing particles, to which additives or other substances may have been added, and where a weight fraction of ≥1% particles have: a) all sizes 100 nm ≤ × ≤ 5 mm; b) for fibres, a length of 300 nm ≤ × ≤ 15 mm and a length/diameter ratio >3", further specifying that "polymers that occur in nature that have not been chemically modified (other than by hydrolysis) are excluded, as are polymers that are (bio) degradable." Another recent definition added "chemically modified natural polymers" to the MPs definition (European Commission, 2024), adding to MPs also not closely plastic materials. MPs are widespread in natural environments worldwide due especially to their persistence, mobility and small size, which increase their potential for long-distance transport through various environmental matrices (Allen et al., 2019; Liu et al., 2019) and direct or indirect assimilation by organisms (Assas et al., 2020; Devereux et al., 2021; Di Lorenzo et al., 2023; Jahan et al., 2019; Romeo et al., 2015; Ugwu et al., 2021). Negative impacts on health and their presence in human body have also been demonstrated (Barceló et al., 2023; Cox et al., 2019; Kannan and Vimalkumar, 2021). MPs can also serve as vectors for various environmental contaminants, including heavy metals, pesticides, persistent organic pollutants (POPs), bisphenol A (BPA), chemicals, and antibiotics, thus compounding their potential ecological and health impacts (Cheng et al., 2023; Rochman et al., 2013; Selvam et al., 2021; Wanner, 2021; Zhou et al., 2019).

Recent studies have also raised concerns regarding microfibre (MF) pollution (Athey and Erdle, 2022; Dris et al., 2016; Finnegan et al., 2022; Hasenmueller et al., 2023; Okyere et al., 2025; Stanton et al., 2019), which include fibres shorter than 5 mm in length. These originated from various sources, including textile production and use throughout their entire life cycle, personal care products such as wet wipes and face masks, cigarette filters, as well as other manufactured fibrous products (Akyildiz et al., 2022; Athey and Erdle, 2022). Anthropogenic fibres are categorized as natural, regenerated (or man-made cellulosic - MMC, or artificial), and synthetic (Finnegan et al., 2022; Stanton et al., 2019). Natural (e.g. cotton, wool) and regenerated (e.g. rayon, viscose) fibres are an accessible source of carbon for microorganisms (Zambrano et al., 2019), have similar biodegradation processes (Park et al., 2004), and, therefore, should not be considered MPs. Moreover, synthetic fibres under 5 mm are MPs. However, textile fibres often consist of mixed natural and synthetic materials or mixed plastics (copolymers). MFs have been widely detected across different environments and matrices (Finnegan et al., 2022; Miller et al., 2017; Okyere et al., 2025; Santonicola et al., 2025; Suaria et al., 2020a, 2020b), as well as in animals (Kim et al., 2021; Le Guen et al., 2020; Remy et al., 2015; Watts et al., 2015; Zhao et al., 2016) and even in human organs (Pauly et al., 1998; Radev, 2026), with greater prevalence of natural and regenerated MFs respect to synthetic ones (e.g. Balestra et al., 2024a; Stanton et al., 2019). Due to the dyes, additives, and chemicals used in their manufacturing they can be dangerous and harmful for ecosystems and species, such as MPs, and can remain in the environment for long periods (Athey and Erdle, 2022; Jemec et al., 2016; Kim et al., 2021; Lusher et al., 2013; Watts et al., 2015).

Given their potential impact on ecosystems, the simultaneous analysis of both MPs and other anthropogenic microparticles (AMPs), especially MFs, is gaining importance in environmental research. Although studies on these micropollutants, particularly MPs, have expanded recently, they focused especially on marine environments and biota (e.g. Cutroneo et al., 2020; Leistenschneider et al., 2021; Ugwu et al., 2021). Terrestrial environments remain poorly studied, such as karst areas, despite their high pollution risk.

Karst areas encompass the major cave systems worldwide and represent ecologically and hydrologically significant environments (Culver and Pipan, 2019; Mammola, 2019; Moldovan et al., 2020). Karst systems are very susceptible to environmental changes and contamination by surface pollution (Gillieson, 2011; Jeong et al., 2023; Kurwadkar et al., 2020; Oppeltová et al., 2024; Ruggieri et al., 2017; White, 1988), due to the surface and subterranean environment connections, such as shafts, stream sinks and caves (Chiarini et al., 2022), which facilitate the fast transport of pollutants even for long distances (Worthington and Ford, 2009). Even if groundwaters and subterranean environments are generally less vulnerable to contamination by emerging contaminants than surface habitats and watercourses, once contaminated are really difficult to treat (Reberski et al., 2022; White et al., 2019). Therefore, monitoring karst systems is fundamental, particularly regarding the presence of micropollutants which can contaminate waters and threat habitats and species.

In karst systems, the presence of MPs and other AMPs is linked to direct and indirect human activities, and is strongly influenced by aquifers hydrodynamics, geological features, karstification degree, local meteorological conditions, and air circulation (Balestra et al., 2023, ; Christman, 2019; Jablonsky et al., 1993). In caves, direct sources of contamination can be linked to tourism in show caves and the presence of speleologists and researchers in the speleological caves, or litter. Tourists, speleologists, and researchers can introduce a variety of allochthonous materials into cave environments, including dust and fibres originating from clothing, footwear, bags, and caving equipment (Balestra and Bellopede, 2022 ; Jablonsky et al., 1993; Valentić et al., 2022). Indirectly, pollution in underground environments can originate from litter, landfill, wastewater discharge, agriculture, transports, industrial production, surface water and soil pollution, and athmospheric deposition on the surface (e.g. Allen et al., 2019; Balestra et al., 2026; Liu et al., 2019; Oppeltová et al., 2024; Zhou et al., 2021). Once in the soil, these materials can be reduced in dimension and transported especially though leaching in soil pores and rock fractures, which involves their movement into deeper soil layers, accumulating in subterranean environments and waters (Chia et al., 2021; Fahrenfeld et al., 2019; Frei et al., 2019; Lwanga et al., 2017; McGechan, 2002; Pérez-Lucas et al., 2018; Viaroli et al., 2022; Wanner, 2021). Biological, chemical, and physical processes can control pollutants migration into underground environments and waters too (Stoppiello et al., 2020).

To date, only a limited number of studies have investigated MP and MF pollution in caves (Piccardo and Bevilacqua, 2024), focusing mainly on sediments and water samples, in unexplored (Balestra and Bellopede, 2025; Luqman et al., 2025), speleological (Balestra et al., 2024a, 2024b; Mutshekwa et al., 2025; Piccardo et al., 2025; Valentić et al., 2022) and show caves (Balestra and Bellopede, 2023; Fraissinet et al., 2025). However, scientific literature on this topic is mostly focused on show

caves, as they offer easier access forresearchers and present relatively minor logistical difficulties for fieldwork. Show caves can play an important role in pilot studies and advancing knowledge on underground pollution, testing new methods and analyzing preliminary data to verify the health of karst system (Balestra and Bellopede, 2022, 2023; Balestra et al., 2023; Piano et al., 2022). Few research has taken into account the entire karst system, from surface to underground environments (Balestra et al., 2023, 2026), and, currently, little is known about contamination sources and potential transport routes in caves. Dripping water in caves can be a significant source of pollution, as water infiltrates through land that may already be contaminated. Into the soil, an accumulation of micropollutants can occur, increasing the possible contamination of water. However, to date, percolation water has never been specifically analyzed.

Evaluating MPs and other AMPs in caves can provide important insights for the management and conservation of karst systems, especially for water, highlighting how indirect pollution can contaminate subterranean environments. In this study we investigated for the first time dripping waters in the Torri di Slivia karst cave, Friuli-Venezia-Giulia, Italy. The aims of this study are: i) to examine, for the first time, the presence of AMPs in cave dripping waters; if detected, ii) to assess their abundance and characteristics, probable sources, potential risks and ecological impacts that such pollutants may pose to karst environments. This study represent the first preliminary assessment of MP and other AMP pollution in cave dripping waters, with the aim of promoting effective conservation strategies for karst areas, which integrate both surface and subterranean components, especially for water resource protection.

2. Materials and method

2.1. Study area

The Torri di Slivia show cave, called also Pejca v Lazcu in Slovenian (22-39VG) is located in the Duino-Aurisina municipality, Friulia-Venezia-Giulia Region, Italy, in the Italian sector of the Classical Karst Region, at about 109 m a.s.l (Fig.s 1–3). The cave has two entrances, an artificial one for tourists and a big natural pit of about 30 m (Figs. 2 and 3). It develops for about 553 m in Mesozoic (Cretaceous) carbonate rock, (Sežana formation), primarily massive and fractured limestones (Jurkovšek et al., 2016), with a total drop of 101.5 m.

The Classical Karst Region (Kras/Carso) is a limestone plateau of approximately 750 km^2 spanning NE Italy (Friuli Venezia Giulia region) and SW Slovenia. It extend for about 15-20 km in width and about 40 km in length in the SW-NW direction (Visintin and Cucchi, 2010; Zini et al., 2022). The region gives its name to global karst terminology, as the first karst phenomena studies originated here (Gunn, 2004). The plateau is primarily composed of Cretaceous to Eocene carbonate rocks (Comeno/Komen Unit) (Cucchi et al., 1987; Placer, 1981), and is heavily karstified, exhibiting a large number of surface morphologies with more than 4500 dolines and over 3500 known caves in the Italian sector (Zini et al., 2015). Due to their high quality and abundance, the waters of the Classical Karst have historically supported the regional economic and social development, and are still tapped for drinking water supply by both the Italian and Slovenian aqueduct systems, serving a population of over 250,000 people (Zini et al., 2010, 2014). However, extensive human intervention has significantly and irreversibly altered this area and the system hydrology (Fornasir, 1929; Gemiti, 2004; Marocco and Melis, 2009). Urbanization, commercial and industrial activities have heightened the vulnerability of these resources in recent decades (Zini et al., 2010).

Different zones of this area are now under environmental protection, however, karst systems are open and close to urban zones, industrial areas, railways and highways, posing ongoing threats to habitats, species and potable waters. Torri di Slivia cave is located in an area frequently visited by individuals and groupsopens for recreational activities (running, cycling, walking, etc.), near the right carriageway of the Carso highway E70 (Trieste – Monfalcone direction), crossed by the railway of the Trieste – Monfalcone line, and near the Provincial Road SP1 (Fig. 3). Moreover, in the past, sinkholes in this area were often used as landfills, and the ones above the cave are no exception. Several buckets of paint had also been poured into this area in the past, especially near the show cave entrance. Consequently, this cavity results a perfect natural underground laboratory for the study of indirect contaminations by MPs and other AMPs.

2.2. Field sampling

Seven sampling points at different distance from the entrance were defined in Torri di Slivia cave (Table 1, Fig. 2), based on the environmental characteristics and hydrology of the caves and on the surface morphologies. All sampling points were located in the stretch of cave

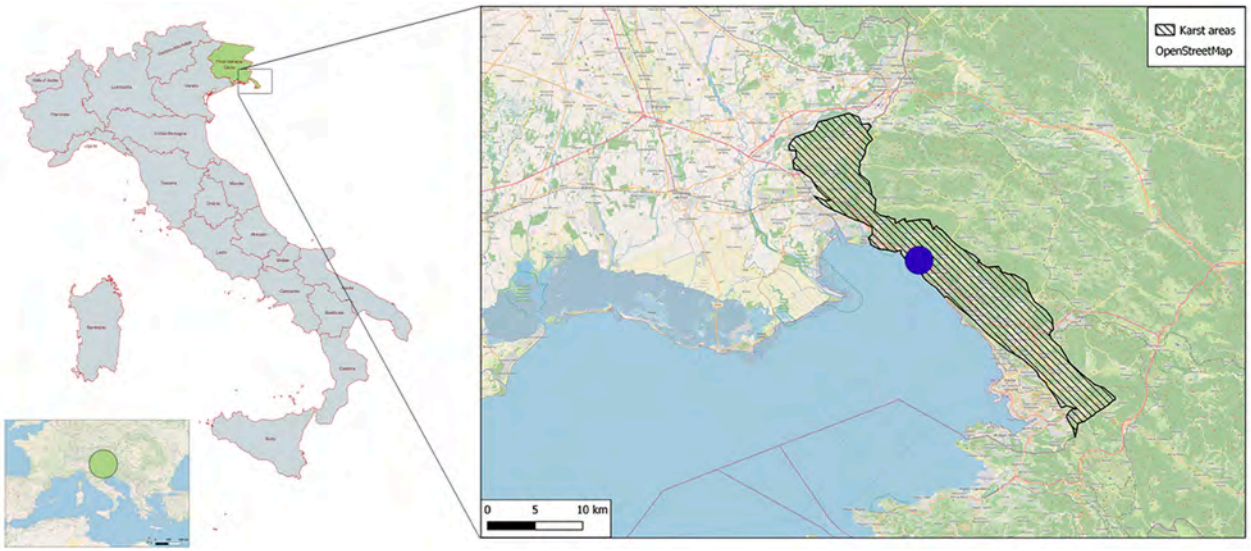


Fig. 1. Study area in the Italian sector of Classical Karst, Friuli-Venezia-Giulia Region. Karst area in black hatch, Torri di Slivia cave in blue circle (Italy map created with mapchart.net, Europe and detail of Italian Classical Karst created with QGIS Desktop 3.12.1 with GRASS 7.8.2 using OpenStreetMap map, modified - openstreetmap.org/copyright).

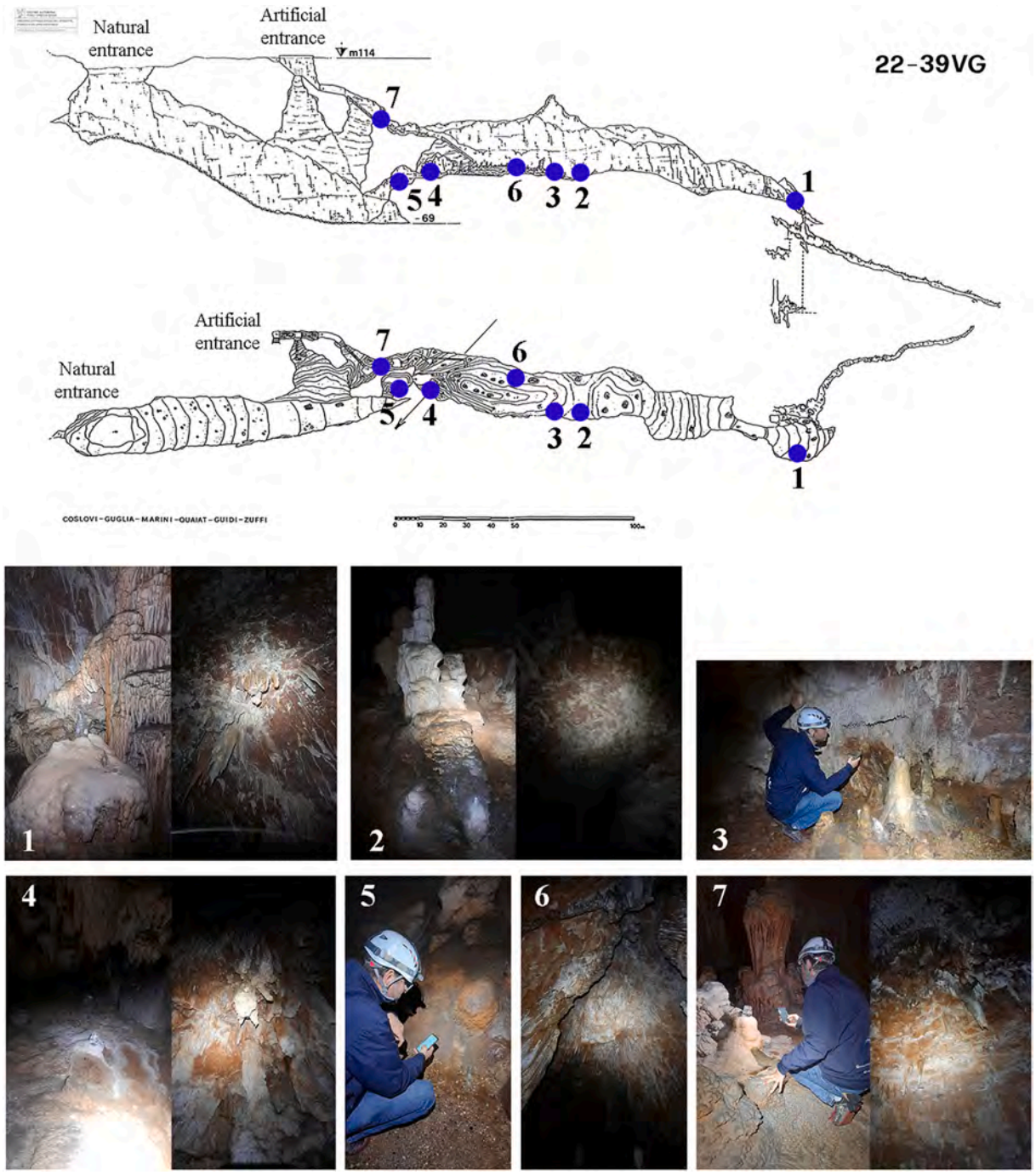


Fig. 2. Torri di Slivia cave surveys with sampling points (blue circles) (Cave surveys by CGEB - Commissione Grotte Eugenio Boegan from https://catastogrotte.regione.fvg.it/scheda/22-Grotta_delle_Torri_di_Slivia, photos by V. Balestra).

between the railway and the highway E70 (Fig. 3).

Samples were named with "Slivia" and the number of the sampling point (from 1 to 7). Monitoring was conducted at the end of March 2025, after an important rainy event.

Despite cave ecological importance and the necessity to understand sources and transport mechanism of MPs in underground environments and groundwaters, these environments are still little studied. Therefore, even if the limited availability of dripping water and extreme conditions in underground environments can complicate analysis and monitoring efforts, any sampling and subsequent analysis are key to improving our understanding of ecosystem health and the indirect impacts of anthropogenic activities.

Water bulk samples (Hidalgo-Ruz et al., 2012) were collected using nitrile gloves, dropping water inside glass jars pre-cleaned with bi-filtered EMSURE ethanol absolute (Supelco, Sigma-Aldrich) and MilliQ water. Glass jars were left on site for about 20 min and then immediately closed with the cap. Blank control was performed using one empty glass jar, placed near the sampling point, for the same time. The jars were coated with anti-impact material to transport them safely in a backpack. Samples were refrigerated at 6 ∘C until analysis in the laboratory.

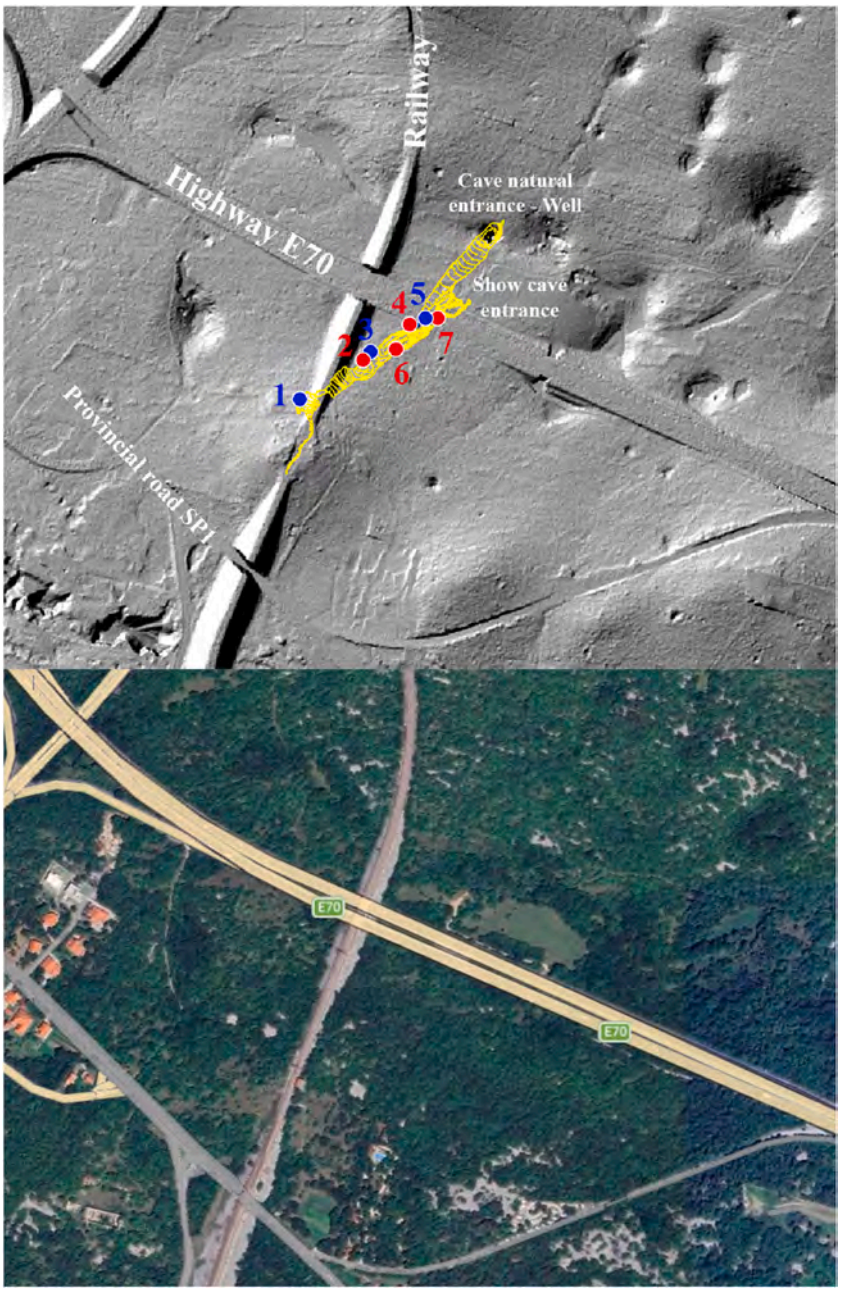


Fig. 3. Sampling points with microplastic pollution respect to surface human activities (Digital Terrain Model). Torri di Slivia cave survey in yellow, sampling points with microplastics and/or other anthropogenic materials in red circles, sampling points without pollution in blue circles (Above: Digital Terrain Model from Regione Autonoma Friuli Venezia Giulia Lidar survey 2017-2020, modified; below: Google Maps, Immagini ©2026 Airbus, Immagini ©2026, CNES/Airbus, Maxar Technologies, Dati Cartografici ©2026, accessed 08/04/2026 h 18:37).

2.3. Laboratory analysis

2.3.1. Contamination control

Where possible, plastic laboratory equipment was replaced with glass and/or metal alternatives. During all lab procedures, researchers wore nitrile gloves and white cotton lab coats. To minimize contamination by airborne AMPs, all open containers, glass objects and equipment were covered with aluminum foil during analysis. Laboratory surfaces and materials were thoroughly cleaned using absolute ethanol and MilliQ water filtered twice. All analyses were conducted under a fume hood.

Blank controls on reagents and water were performed on 30% H_2O_2 (Merck), EMSURE ethanol absolute (Supelco, Sigma-Aldrich), and MilliQ water to evaluate potential AMP contamination. They were filtered twice through a 1.2 μm glass fibre filter (Enrico Bruno srl).

Blank control on air was performed using one open pre-cleaned Petri dish during the analysis. Once the analysis was completed, the petri was washed with bi-filtered MilliQ water and filtered using the same procedure as the other samples. The blank correction method applied in this study follows Shruti and Kutralam-Muniasamy (2023) and references therein, whereby the contribution of pollutants identified in the blanks is subtracted from the results of the analyzed samples.

2.3.2. Dripping water analysis

Water samples were analyzed following the previous procedures outlined for water samples in caves and karst systems (Balestra et al., 2024a, 2024b), adapted. The collected water samples were subjected to organic matter removal (OMR) using an amount of 30% H_2O_2 solution

Table 1
Characteristics of the sampling points and collected samples.

Sampling point	ml	Drops/ minute	Characteristics of the sampling point
Slivia 1	102	30	End of the cave, in the oldest and not-touristic part of the cavity. Water was collected under a wall covered by red and white stalactites. Dripping water creates a large stalagmite.
Slivia 2	64	14	At the end of the tourist route, under a white stalagmite. The most distant point from the ceiling. Slow dripping.
Slivia 3	136	31	At the end of the tourist step with the anti-slip mats. Water was collected from a fast dripping from the low wall.
Slivia 4	140	106	Fast dripping from stalactites on the ceiling. The dripping water creates different rimstone dams.
Slivia 5	16	4	At the end of the tourist steps, in a pit with a gavel bottom. Slow dripping near a calcite flow.
Slivia 6	129	94	Under the roost of the bat colony, near big dams.
Slivia 7	132	28	At the end of the first tourist step. Water was collected from a fast dripping from the ceiling, creating a stalagmite.

equal to half the sample. Samples were shaken (50 revs/min) for 2 h at 45°C in a Shaker Water Bath SBS30, Bibby Stuart Scientific. Once cooled, waters were then filtered through a 1.2 μm pore size silver filter (GVS Life Sciences, Membrane Disk 47 mm) using a glass vacuum pump. Filters were placed in glass petri dishes, coated with aluminum foil, and dried in an oven at 40°C until completely dry.

2.4. Microparticle identification and characterization

A combination of microscopic and spectroscopic techniques was employed for a more comprehensive study of MPs and other AMPs.

Microparticles retained on filters were initially examined under a Leitz ORTHOLUX II POL-MK microscope equipped with a 12 Mpx DeltaPix Invenio 12EIII Camera. AMPs were counted at 2.5× magnification and observed at 10× or higher magnifications for identification and characterization. This step followed the stringent selection criteria reported in previous research (Crawford and Quinn, 2016; Hidalgo-Ruz et al., 2012; KIMO Sweden, 2007). For MF analysis, detailed morphological comparison was carried out using longitudinal and x-sectional images of natural, regenerated and synthetic fibres (e.g. Khan et al., 2017). Each filter was examined under visible light and, in parallel, under a UV 365 nm flashlight (Alonefire SV10, 5W), by exploiting fluorescent additives used in many plastics and textiles (Balestra and Bellopede, 2025; Luo et al., 2019; Qiu et al., 2015). Microparticles between 0.05 and 5 mm were analyzed. Microparticles that could not be confidently identified as MPs or other AMPs were excluded from the analysis. Identified MPs and other AMPs, especially MFs, were characterized according to the Standardised size and colour sorting system (SCS) (Crawford and Quinn, 2016).

Subsequently, spectroscopic analysis on microparticles found on the filters were performed with the micro-Fourier Transform Infrared Spectroscope (μFTIR) Nicolet iN10MX, Thermo Fisher Scientific, in reflection mode, within the spectral range of 4000 to 650 cm^{-1}, with 24 scans taken per sample. The spectra were analyzed with the Omnic Picta software and verified visually by the operator. Only spectra with a match degree ≥ 70% were accepted.

3. Results

3.1. Abundance and composition

Based on the values obtained from the blank samples, potential airborne contamination linked to sampling in cave turned out to be about 21 items/sample, and 13 items/sample for laboratory activities, all not synthetic.

MPs were found in three of seven sampling areas, distributed in all the cave: Slivia 2, 4, 6 (Tables 2 and 3 and Fig.s 3, 4). The value was estimated from 0 to 23 MP/L of dripping water, with a mean and standard deviation of 6.6 ± 8.7 MP/L (Table 2). These sampling points were located in the stretch of cave between the railway and highway E70 (Fig. 3). In particular, Slivia 4 sampling point was exactly under the highway E70 border, and Slivia 2 near the railway (Fig. 3).

Considering also natural and regenerated materials of anthropogenic origin, four monitored sampling points result polluted: Slivia 2, 4, 6 and 7 (Table 2, Fig. 4). The total quantities of AMPs found in the sampling points were estimated from 0 to 143.9 items/L, with a mean and standard deviation of 33.8 ± 50.6 items/L (Table 2). Slivia 7 sampling point resulted exactly under the highway E70 (Fig. 3).

Considering the amounts found for each sampling point, Slivia 7 was the most polluted, however, Slivia 6 is the only point with both MPs and natural and regenerated MFs of anthropogenic origin and the one with the major number of found MPs (n = 3). Slivia 1, 3 and 5 were not polluted. There is no direct correlation between the amount of percolating water and the number of detected items (AMPs: rs = 0.28 p = 0.53; MPs: rs = 0.12 p = 0.84) (Table 2).

Microscopic and spectroscopic analysis showed that 80.5% of AMPs were natural and regenerated materials of anthropogenic origin, and 19.5% synthetics. Of the not synthetics, all materials were cellulosic, except for one woolen MF found in Slivia 7. μFTIR analysis with good spectra (>70%) confirmed the presence of polyethylene terephthalate (PET) in Slivia 4 (n = 1) and 6 (n = 3), and polyurethane (PU) in Slivia 2 (n = 1) (Table 3, Fig.s 3, 4).

3.2. Shape and size

Fibre-shape dominated all samples (81.6%), followed by fragments (17.1%) and film (1.3%) (Table 4, Fig.s 5A, B). All MPs were fragments (n = 4), except for one PET fibre. Of the other AMPs, 94% were natural and regenerated MFs.

Big AMPs (5-1 mm) accounted for only 10.7%, while AMPs < 1 mm were the most abundant (89.3%) (Table 4, Fig.s 5C, D). Most microparticles had size between 0.49 and 0.10 mm (51.5%) (Table 4, Fig.s 5C, D).

3.3. Fluorescence and colour

The 90.9% of the analyzed AMPs were fluorescent under UV light, of which the major part had blue fluorescence (78.4%) (Table 4, Fig.s 5 E, F).

Of the fluorescent AMPs found 90.8% were transparent, followed by beige (11.5%), green (3.7%) and blue (3.4%) ones; particles with other colours had percentages between 2 and 1.4% (Fig. 5G). Non-fluorescent AMPs were mainly beige (36.6%), black (31.7%), and grey (19.8%); particles with other colours had percentages of 5.9% (Fig. 5H).

4. Discussion

4.1. Dripping waters in cave can transport microplastics and other microparticles of anthropogenic origin

MPs were found in three out of seven sampling points (Slivia 2, 4 and 6) (Table 2, Fig. 3). Taking into the account also natural and regenerated microparticles of anthropogenic origin, even Slivia 6 and 7 resulted polluted (Table 2, Fig. 3). Sampling points Slivia 1, 3 and 5 had no particles (Table 2, Fig. 3).

To the authors knowledge, this is the first research about MP and other AMP pollution in cave dripping waters. Therefore, comparisons with other studies are not possible at the moment. Nevertheless, some

Table 2
Anthropogenic microparticle (AMP) abundances for each cave dripping water sample in Torri di Slivia cave, and estimates for 1 L of water. AMPs were divided into synthetic (microplastics) and non synthetic (natural and regenerated). Reported values were corrected by subtracting the values found in the blanks.

Sampling point	ml	Not synthetic	Synthetic	TOT	Not synthetic/L	Synthetic/L	TOT/L
Slivia 1	102	0	0	0	0.0	0.0	0.0
Slivia 2	64	0	1	1	0.0	15.6	15.6
Slivia 3	136	0	0	0	0.0	0.0	0.0
Slivia 4	140	0	1	1	0.0	7.1	7.1
Slivia 5	16	0	0	0	0.0	0.0	0.0
Slivia 6	129	6	3	9	46.5	23.3	69.8
Slivia 7	132	19	0	19	143.9	0.0	143.9
SUM		25	5	30			
MEAN		3.6	0.7	4.3	27.2	6.6	33.8
St. dev.		6.6	1.0	6.7	50.3	8.7	50.6

Table 3
Microplastic (MP) characteristics in the Torri di Slivia cave dripping water.

Sampling point	Shape	Size [mm]	Colour	Fluorescence	μFTIR
Slivia 2	Fragment	0.48	Beige	Yellow	Polyurethane
Slivia 4	Fragment	0.15	Transparent	Blue	Polyethylene terephthalate
Slivia 6	Fragment	0.17	Beige	Green	Polyethylene terephthalate
Slivia 6	Fibre	0.08	Green	Green	Polyethylene terephthalate
Slivia 6	Fragment	0.11	Beige	No	Polyethylene terephthalate

assumptions can still be drawn regarding the presence of MPs and other AMPs in caves. However, it must be taken into account that different research used diverse methods, the size range of detected microparticles are not the same, some studies include anthropogenic MFs in the abundances, others separate MPs from MFs, and examined caves have different environmental and climatological conditions, and have diverse possible anthropogenic sources.

Pollution in the karst cave dripping waters can come from the surface anthropogenic activities, rubbish buried, atmospheric depositions, and water infiltrations through fractures and soil. Torri di Slivia cave is crossed on the surface by the highway E70 and the railway (Fig. 3), and is located near the Provincial road SP1, therefore, the presence of MPs and other AMPs may be linked to traffic, road markings, road wear, tire degradation, as well as dust and litter (Andersson-Sköld et al., 2020; Burghardt and Pashkevich, 2023). Moreover, unfortunately, sinkholes near the cave were used as landfills times ago and several buckets of paint had also been poured into the area, especially near the tourist cave entrance. Slivia 2, 4, 6 and 7 sampling points result located in the stretch of cave between the railway and the highway E70 (Fig. 3). In particular, Slivia 2 is near the railway, Slivia 4 is exactly located under the highway border, and Slivia 7, the most polluted, under the highway (Fig. 3). Therefore, pollution in dripping water could be linked to percolation from this road drains. However, Slivia 6, the most polluted by MPs and the only sampling point with both MPs and MFs, is distant from both. Dripping water from this sampling point comes from a very high point of the ceiling, which is characterized by the presence of a colony of bats with thousands of individuals, which could unintentionally carry MPs and MFs in the upper part of the cave, contaminating also dripping waters. Moreover, even Slivia 1, 3 and 5 are near the highway and the railway, however, they were not polluted (Fig. 3). However, no definitive conclusions can be drawn regarding the origin of these contaminants. Multiple sources should be considered as plausible contributors, such as agriculture, waste, and soil-water-air contamination, among other potential inputs. Consequently, subsequent studies will be done to better understand the possible sources of pollution and water routes from the surface to the cave.

Microscopic and spectroscopic analysis showed that 80.5% of AMPs were natural and regenerated materials of anthropogenic origin (19.5% synthetics), and that fibre-shape dominated all samples (81.6%), followed by fragments (17.1%). Inside the Cliff cave, US, 31% of particles was cellulosic and 29% plastic (Hasenmueller et al., 2023). In natural environments, different research reported an higher abundance of non-synthetic MFs compared to synthetic ones, with cellulosic fibres, particularly cotton, being the most prevalent (Athey and Erdle, 2022). In the Italian sector of the Classical Karst, the monitoring of MF pollution in waters of springs and caves highlighted that 64.4% was not synthetic and only 13.4% was MPs (Balestra et al., 2024a). The high presence of fibres in Torri di Slivia cave suggests that pollution in this area could be linked mainly to textiles deterioration. MFs could be transported in the area by atmospheric deposition or could be linked to rubbish buried and geo-textiles deterioration from the highway and the railway built near the cave. Fragments may have originated from the breakdown of waste in the environment or from nearby human activies. These data could confirm the presence and accumulation of MPs and MFs in karst environment over time. In a karst system, pollution may spread more slowly compared to other environments, particularly when transported by water, due to the specific type of karst present in the area. As a result, pollutants may accumulate in rock fractures within the system, reaching the cavity at a later time, for example through dripping waters.

Analysis on MPs in the Torri di Slivia cave showed the presence of PET and PU. All MPs resulted fragments (n = 4) except one fibre of PET. PET is one of the most used polymers in the world, with main applications in the packaging, textile and technical-industrial sectors. As packaging, it is mainly used for food and beverage, especially for bottles and containers, due to its light weight and durability. It is also widely used in the production of synthetic fibers, as well as in technical applications such as thermoformed films and trays. PU is a very versatile material with numerous applications in different sectors. Its main applications include thermal insulation in construction, the production of mattress and padding foams, components for the automotive industry, footwear soles, coatings, adhesives and sealants. Moreover, PU is one of the plastic often contained in the paints for automobile or metals. PET and PU were previously found also in the waters of Bossea cave, Trebiciano cave, Mariano well, Postojna cave system, Kačna cave and Škocjan Caves system, as well as in the sediments of some of these caves (Balestra et al., 2023, 2024b; Valentić et al., 2022).

AMPs < 1 mm (up to 0.05 mm) were the most abundant (89.3%), of which the major part had size between 0.49 and 0.10 mm (51.5%). However, only particles between 5 and 0.05 mm were analyzed in this research, therefore, micropollutants with smaller dimensions could be present and in larger quantities.

As reported in recent research carried out in karst systems and caves (Balestra et al., 2023, 2024a, 2024b), the majority of the AMPs found in Torri di Slivia cave were fluorescent under UV light (90.9%). These data indicate that UV light to identify AMPs is an effective good method, though it is not enough for a thorough particle analysis. Data about AMP fluorescence could provide valuable information about their consumption by organisms, as well as the colour. The accumulation of MPs and

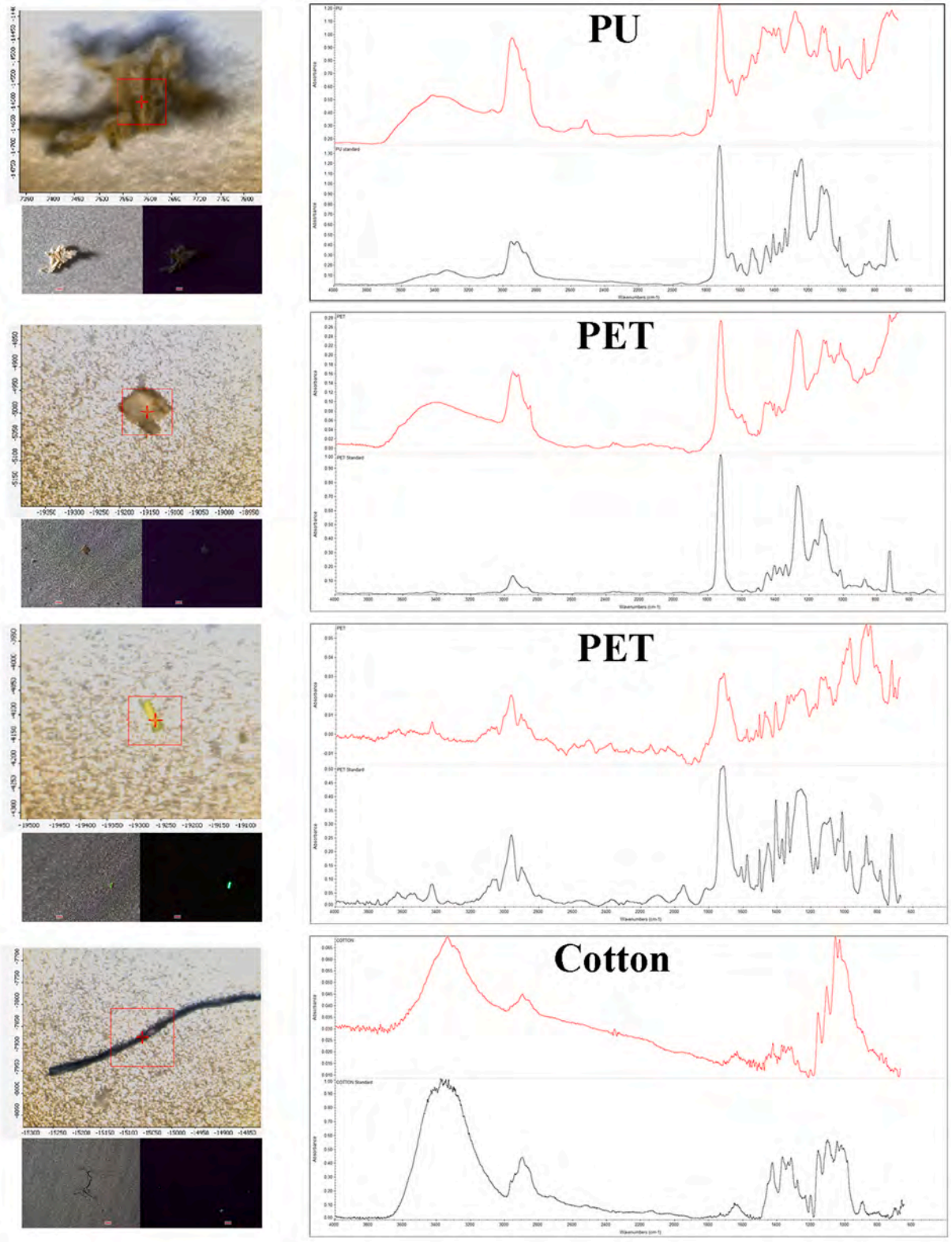


Fig. 4. μFTIR spectra of selected microparticles of anthropogenic origin found in the dripping water of the Torri di Slivia cave, Italy., together with their corresponding optical microscope images acquired under both visible and UV light. Images: μFTIR particle visualization at the top, optical microscope images at the bottom left, and optical microscope images under UV light at the bottom right.

MFs poses a serious threat to ecosystem health, as many organisms ingest them unintentionally, mistaking them for food sources (Devereux et al., 2021; Gomiero et al., 2018; Kokalj et al., 2025; Pukos et al., 2023; Sforzi et al., 2024). Once into the food webs, AMPs may affect higher trophic levels, potentially triggering cascading ecological consequences (Zhang et al., 2023). Previous studies have shown that the color of anthropogenic microparticles (AMPs) can provide valuable information on ingestion patterns between various organisms, making this parameter

Table 4
Anthropogenic microparticle characteristics found in the dripping water of the Torri di Slivia cave.

Sampling point	ml	Items	Items/L	Fluorescence		Fluorescence colour				Size				Shape		
				Fluorescent	Not fluorescent	Blu	Red	Green	Other	5-1 mm	0.99-0.50 mm	0.49-0.10 mm	0.09-0.05 mm	Fiber	Fragment	Film
Slivia 1	102	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Slivia 2	64	1	15.6	1.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0	1.0	0.0
Slivia 3	136	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Slivia 4	140	1	7.1	1.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	1.0	0.0
Slivia 5	16	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Slivia 6	129	9	69.8	7.0	2.0	5.0	0.0	2.0	0.0	1.3	1.3	5.1	1.3	7.0	2.0	0.0
Slivia 7	132	19	143.9	18.2	0.8	17.5	0.8	0.0	0.0	1.9	4.6	8.4	4.2	17.5	1.1	0.4
SUM		30.0		27.3	2.7	23.5	0.8	2.0	1.0	3.2	5.9	15.4	5.5	24.5	5.1	0.4
MEAN		4.3	33.8	3.9	0.4	3.4	0.1	0.3	0.1	0.5	0.8	2.2	0.8	3.5	0.7	0.1
St. dev.		6.7	50.6	6.3	0.7	6.0	0.3	0.7	0.3	0.7	1.6	3.0	1.5	6.2	0.7	0.1

ecologically relevant (Carpenter et al., 1972; Jahan et al., 2019; Lusher et al., 2013; Romeo et al., 2015; Ugwu et al., 2021; Zhao et al., 2016). In karst systems, blue and fluorescent AMPs were found in the digestive tract of specialized crustaceans of Italian caves and springs (Sforzi et al., 2024). In the same study, blue, transparent, and black particles were found with high amounts in all analyzed superficial and subterranean waters. In our study, transparent particles were found in the highest amounts, as in Cliff cave, US, and in different Italian caves (Balestra and Bellopede, 2023; , 2024a, ; Hasenmueller et al., 2023). Scientific studies have also highlighted the relationship between MP color and their affinity for chemical pollutants, which may enhance their role as contaminant vectors (e.g. Frias et al., 2010; Karapanagioti et al., 2011). In particular, yellow and black MPs have been associated with higher levels of adsorbed pollutants in marine environments.(Frias et al., 2010; Karapanagioti et al., 2011).

MP pollution in karst systems, as well as MFs and other AMPs, pose a significant threat to water quality, ecosystem health and biodiversity, making this issue critical to conservation efforts. Monitoring these contaminants in dripping waters of underground karst systems is essential to assess the indirect impacts of human activity, improve understanding of pollutant sources and transport dynamics, and support the development of targeted conservation strategies for these vulnerable ecosystems.

Fieldwork in cave environments presents significant logistical and ecological challenges. Large-volume sampling is often unsuitable, due to the fragility of these environments, logistical difficulties, and unstable terrain whichcomplicates sampling and transportation. Despite these limitations, underground environments remain under-explored, therefore, new data are critical to deepening ecological understanding and identifying potential threats. Show caves, due to their accessibility, can serve as valuable sites for testing methodologies, monitoring pollutants and environmental processes, such as MP pollution and leaching. Increased efforts to sample and study karst systems are critical, and conservation strategies must recognize ecological connectivity between surface and subsurface karst environments. In this perspective, promoting collaborations with cavers to facilitate access to otherwise inaccessible subterranean environments can be instrumental in acquiring new and valuable data. Such efforts are essential to advance research in this field and to develop targeted protection and mitigation strategies for these isolated and ecologically sensitive systems. Driving political change is essential to advance effective plastic reduction strategies and incentivize the adoption of sustainable materials in production. Education rooted in environmental sustainability principles aimed at society plays a crucial role in the conservation and management of water resources, protected habitats, and biodiversity.

4.2. *Methodological challengesand technicalaspects*

Research on AMPs is hindered by contamination risks, methodological challenges, and sampling and analysis issues.

Contamination of not synthetic AMPs was found in our blanks, highlighting the importance of control airborne and operator-related contamination during sampling and analysis (Scopetani et al., 2020; Shruti and Kutralam-Muniasamy, 2023). Pre-filtering of all used chemicals and MilliQ water was an essential step, and both scientists and manufacturers must be more aware of material-induced contamination to improve data reliability and reduce pollution.

OMR is important for some matrices, however, certain chemicals used in the process may lead to the partial or complete degradation of the AMPs (Athey and Erdle, 2022; Treilles et al., 2020). H_2O_2, though widely used for OMR in MP and MF analyses, can alter the mechanical properties and IR spectra of the samples, increasing the fragility of some plastics and other not synthetic materials (Athey and Erdle, 2022), potentially causing fragmentation and, therefore, an overestimation of pollutants during analysis (Treilles et al., 2020). Groundwater and cave waters tend to have little organic material, however, in this case we had

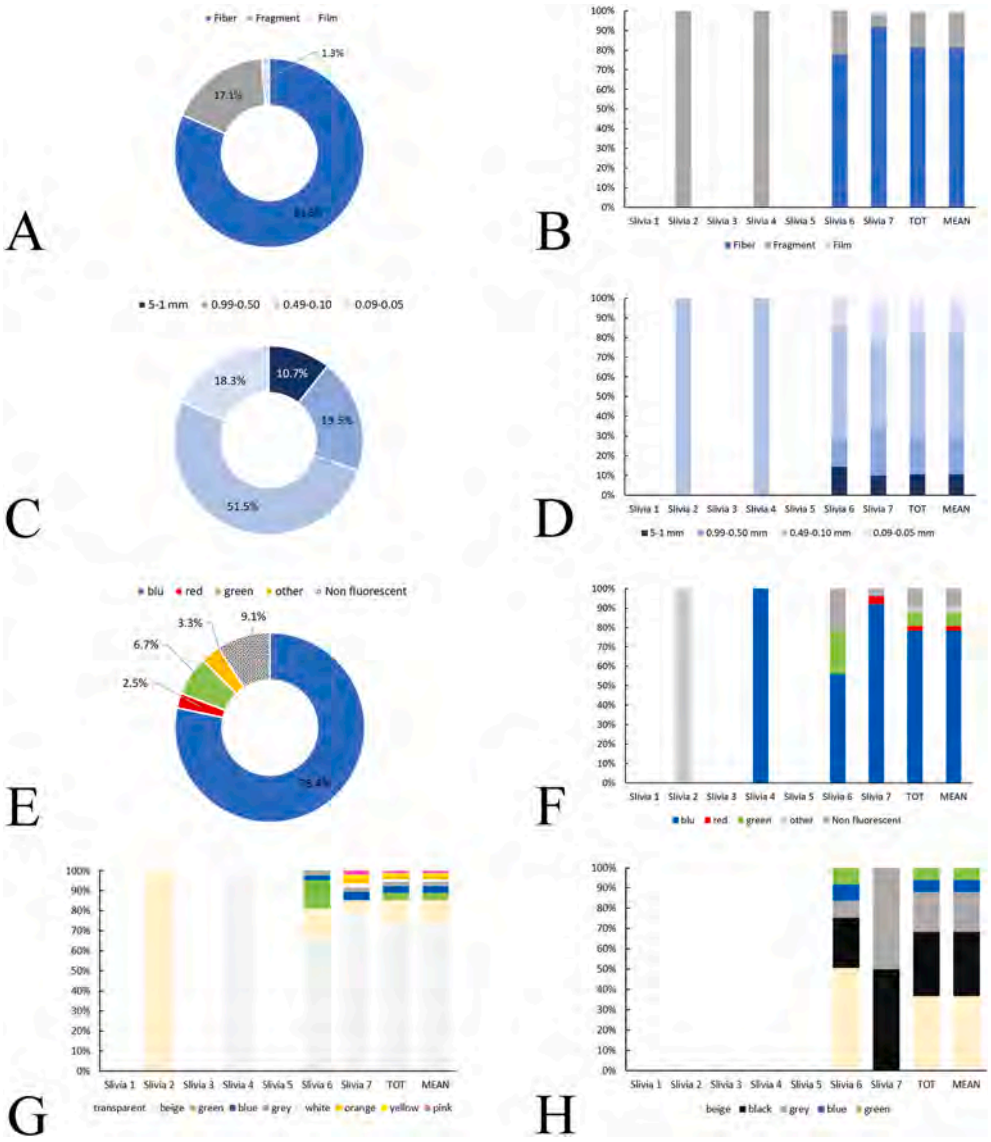


Fig. 5. AMP characteristics in the dripping water of Torri di Slivia cave. A: shape, total; B: shape for each sampling points; C: size, total; D: size for each sampling point; E, fluorescence, total; F: fluorescence for each sampling point; G: colour of fluorescent particles; H: colour of not fluorescent particles.

no previous feedback, so we decided to do OMR anyway. Probably, given the clearness of the percolation waters, a decidedly smaller quantity of H_2O_2 would have been sufficient.

Microscopy is widely used for MP and MFs characterization, due to its cost-effectiveness and allowing to observe color and morphology of the microparticles. However, visual analysis cannot determine chemical composition of the particles and is labor-intensive. Moreover, microscopy is not recommended alone for analyzing small particles (Hidalgo-Ruz et al., 2012; Huang et al., 2023; Song et al., 2015). Preliminary microscopic screening aids in differentiating synthetic from natural or regenerated materials. Spectroscopic techniques are essential to define the chemical composition of identified microparticles, but are time-consuming, require expensive equipment and skilled personnel. Moreover, they face challenges due to complex environmental matrices and surface contamination of microparticles (Song et al., 2015). Therefore, the combination of both techniques was preferred in this work.

The methodologies developed for detecting and characterizing MFs and other not synthetic microparticles of anthropogenic origin were initially designed for MPs, therefore, analysis on these materials are challenging (Athey and Erdle, 2022), spectroscopic analysis included. These materials exhibit lower signal intensities compared to synthetic ones, show dye interference and spectral overlap (Comnea-Stancu et al., 2017; Li et al., 2010; Remy et al., 2015; Zambrano et al., 2019). Consequently, accurate identification requires high-quality spectral matches and a combined methodological approach to improve reliability in environmental AMP detection (Song et al., 2015). Errors in spectral library matching can lead to substantial misinterpretations; therefore, in this study, only spectral identifications with a confidence level above 70% were considered reliable, together with the operator visual check.

5. Conclusion

This study provides new insights into MP pollution and other AMPs, especially natural and regenerated MFs, in karst systems, marking the first record of this kind of pollution in cave dripping waters. The results demonstrate that surface human activity can have an indirect impact on even the dripping waters in underground environments.

This work encourages further investigations in subterranean karst environments, using also show caves to study pollution in these under-researched habitats. Although only five MPs were found in three

samples, and MFs only in two samples, abundances in some sampling points are not low, considering that these data concern percolation waters coming out of fractures in the rock. Therefore, further investigations on cave dripping waters are needed. The predominance of not-synthetic fibres, the shape and composition of identified MPs indicate textiles, illegal landfills in sinkholes, garbage, nearby urban activities, roads and railway as a likely source of pollution.

Given the strong surface–underground connectivity typical of karst environments, a broader monitoring strategy is needed to improve understanding of MP and other AMP sources, transport mechanisms and ecological implications. Long-term multidisciplinary investigations will be key to assessing the extent and impact of these pollutants contamination, ultimately supporting the development of effective conservation strategies for karst ecosystems.

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CRediT authorship contribution statement

Valentina Balestra: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **Raffaella Mossotti:** Formal analysis, Resources, Writing – review & editing. **Luca Zini:** Investigation, Writing – review & editing. **Rossana Bellopede:** Resources, Writing – review & editing.

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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Data availability

Data will be made available on request.

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