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Explorations in the dark continent: Did microplastics and microfibres get here before us?

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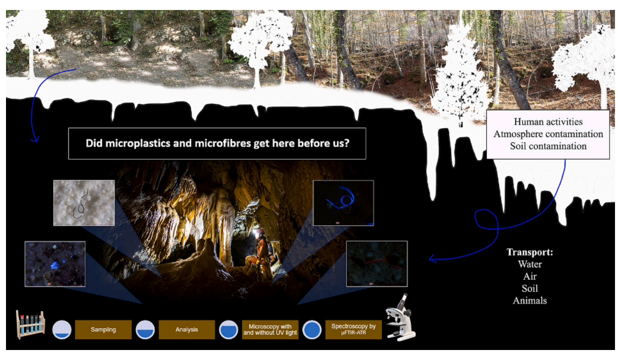
HIGHLIGHTS

- The dark continent is polluted by anthropogenic microparticles.
- Cellulosic microfibres are more abundant than microplastics.
- Small size and fibre shape microparticles dominate the sediment samples.
- Samples contained especially fluorescent and clear microparticles.
- Monitoring unexplored caves is fundamental for karst areas conservation.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastic and microfibre pollution is a global concern, however, karst areas remain understudied. Because of their properties, these anthropogenic microparticles are particularly hazardous, and easily transportable, reaching also remote areas. The underground world, called also dark continent, is a treasure of information, and remained the last frontier of terrestrial exploration: many parts of the underground world have not yet been accessed. In the hypogeal environments, pollution is closely linked to the connections between surface and subterranean habitats, the hydrodynamics of the aquifer, geology, and local environmental conditions. This study aims to investigate, for the first time, the presence of microplastics and microfibres in unexplored caves, revealing how human activity could indirectly impact even the uncontaminated environments of the dark continent. Together with speleologists, we collected and investigated sediment samples from unexplored caves of the Abruzzo Region, Italy. Examined anthropogenic microparticles were counted and characterized by composition, size, shape, fluorescence, and colour, via microscopy and spectroscopy. Microplastic concentrations resulted low or absent, moreover, natural and regenerated microfibres ones were higher. Fibre-shape was the most common. Most of the microparticles were clear and fluorescent under UV light. Pollution sources in this area likely include atmospheric deposition, nearby human activities, roads, and garbage. These results highlight anthropogenic microparticle pollution exists in unexplored karst caves, which could impact subterranean habitats, species, and water resources. Given the link between surface and underground karst environments, more monitoring and protection are needed. This work encourages speleologists to collect samples during explorations

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too, as these rarely studied environments offer crucial insights into karst systems, potential threats, and conservation needs. Future long-term studies will clarify pollutant sources, transport, and effects on ecosystems.

1. Introduction

Microplastics (MPs) pollute natural environments worldwide. The first definition of MPs was introduced by Prof. Richard Thompson and his team in 2004 (Thompson et al., 2004). They defined MPs as plastic particles smaller than 5 mm, resulting from the fragmentation of larger plastic or intentionally produced plastic waste with small dimensions. This definition has been adopted and extended by several scientific and environmental organizations, defining MPs as synthetic particles with a 5 mm–1 μm dimension (e.g. Frias and Nash, 2019; International Organization for Standardization and European Committee for Standardization, 2020). Moreover, after recent scientific considerations, it is preferable to distinguish between MPs (1 mm–1 μm) and big MPs (5–1 mm) (International Organization for Standardization and European Committee for Standardization, 2020). However, the definition of MPs is still much debated. The recent Commission Delegated Decision (EU) 2024/1441 about MPs detection in drinking waters defined MP as "small discreet object that is solid, insoluble in water and is partially or wholly composed of synthetic polymers or chemically modified natural polymers" (European Commission, 2024), and ISO 4484-2 (International Organization for Standardization, 2023) defined MP as "material consisting of a solid polymer containing particles, to which additives or other substances may have been added", adding to MPs also not synthetic materials.

The properties of plastics, such as their resistance, durability, and low weight, make them particularly hazardous pollutants in natural environments. The morphology and size of MPs further increase their harmfulness, as these particles can be easily transported over long distances through various environmental matrices (Allen et al., 2019; Liu et al., 2019). Recently, their presence in human body and negative impacts on health has also been demonstrated too (Barceló et al., 2023; Cox et al., 2019; Kannan and Vimalkumar, 2021). MPs can be assimilated either directly or indirectly by organisms and may be toxic, constituting a serious ecological emergency (Assas et al., 2020; Devereux et al., 2021; Jahan et al., 2019; Romeo et al., 2015). MPs may also contain or adsorb other pollutants, such as pesticides, persistent organic pollutants (POPs), bisphenol A (BPA), chemicals, heavy metals, or antibiotics, which amplifies the environmental risks associated with their presence (Cheng et al., 2023; Rochman et al., 2013; Selvam et al., 2021; Wanner, 2021; Zhou et al., 2019).

Microfibres (MFs) include fibres <5 mm in length, originated from textile production throughout their entire life cycle, cigarette filters, personal care products like face masks and wet wipes, and other manufactured fibrous products (Athey and Erdle, 2022). Anthropogenic fibres are divided in natural, regenerated (or man-made cellulosic - MMC, or artificial), and synthetic (Finnegan et al., 2022; Stanton et al., 2019). Natural fibres derive from the treating of plant (cellulosic) and animal (proteinaceous) fibres, such as cotton or wool. Regenerated fibres are disbanded from cellulose material, such as rayon/viscose. Natural and regenerated MFs are an accessible source of carbon for microorganisms (Zambrano et al., 2019), and biodegradation processes of regenerated MFs are similar to cotton ones (Park et al., 2004), therefore, natural and regenerated fibres should not be considered MPs. Synthetic fibres are made of plastic polymers, therefore, MFs <5 mm can be considered MPs following the original definition of Thompson. Moreover, different fibres used in textile production are copolymer, a mix of different plastics or of cellulosic and synthetic materials, making difficult to recognize them in the three main categories. Recent studies raised concerns about natural and regenerated MFs too (Athey and Erdle, 2022; Balestra et al., 2024a; Dris et al., 2016; Finnegan et al., 2022; Hasenmueller et al., 2023; Stanton et al., 2019). These MFs have been likely included in MPs monitoring by hundreds of research in the past, because they are processed industrially and coloured (Obbard et al., 2014; Woodall et al., 2014), increasing MP concentrations (Wesch et al., 2016). However, non-synthetic fibres can now be included in the definition of MPs as well, as expressed in recent standards and regulations (European Commission, 2024; International Organization for Standardization, 2023).

As MPs, MFs have been detected in different environments and matrices (Balestra et al., 2024a; Finnegan et al., 2022; Stanton et al., 2019; Suaria et al., 2020a; Suaria et al., 2020b), as well as in animal (Le Guen et al., 2020; Remy et al., 2015; Zhao et al., 2016) and human organs (Pauly et al., 1998). Recently studies have shown prevalence of natural and regenerated MFs into the environment compared to MPs (e.g. Balestra et al., 2024a; Stanton et al., 2019), and in the gastrointestinal tract of some animals (e.g. Remy et al., 2015; Zhao et al., 2016), suggesting that these particles can be poisonous for ecosystems as well as MPs, due to the dyes, additives, and chemicals used in their manufacturing (Athey and Erdle, 2022; Kim et al., 2021; Lusher et al., 2013). Under laboratory conditions, negative effects on animal health have been also observed (Jemec et al., 2016; Watts et al., 2015).

Consequently, analyzing both pollutants in environmental research is becoming increasingly important. Despite the recent surge in studying these micropollutants in natural environments, especially MPs, while sea environments have been extensively researched over the years (e.g. Cutroneo et al., 2020; Leistenschneider et al., 2021; Tsang et al., 2017), several environments, such as karst areas, remain poorly studied despite their high pollution risk, and research in this areas is at the initial stage.

Karst areas form the major cave systems worldwide. Subterranean environment are extremely interesting from different perspectives, host aquifers that provide drinking water (Moldovan et al., 2020), and support fragile ecosystems and species, such as troglobiont (i.e. specialist of underground environments) (Culver and Pipan, 2019; Mammola, 2019). Due to the open nature of these systems, they are very susceptible to environmental changes (Balestra et al., 2023; Gillieson, 2011; Kurwadkar et al., 2020; Ruggieri et al., 2017; White, 1988), therefore, subterranean environments are often protected. However, these environments are often subjected to contamination by surface pollution, because of the surface and subterranean environment connections (Chiarini et al., 2022). Despite the ecological and economic significance of these environments, assessments of MP pollution in karst systems are recent and rare (e.g. Balestra et al., 2024b; Balestra et al., 2023; Panno et al., 2019; Re, 2019; Shu et al., 2023; Valentić et al., 2022; Viaroli et al., 2022), and only few research studied natural and regenerated MF pollution (Balestra et al., 2024a; Baraza and Hasenmueller, 2023; Hasenmueller et al., 2023). Therefore, monitoring these habitats is fundamental, particularly regarding the presence of microscopic pollutants, which can harm habitats and species, and contaminate waters.

Anthropogenic microparticles (AMPs) pollution (MPs and natural and regenerated MFs) in karst habitats can be linked to direct and indirect human activities, and is strictly related to the hydrodynamic regime of the aquifer, air circulation, geology, karstification degree, and the local meteorological conditions. Direct contamination in subterranean environments is linked to human presence, such as tourists in show caves or speleologists and researchers in the other ones. The indirect contamination in these environments can come from litter, wastewater, agriculture activities, transports, industrial production, soil and surface water pollution, and atmospheric deposition. The presence of MPs in precipitation has been previously detected in various regions (e.g. Allen et al., 2019; Liu et al., 2019), and is closely linked to soil contamination (Zhou et al., 2021). Once in the soil, MPs can be reduced in dimension and transported through soil pores and rock fractures, accumulating in subterranean waters and environments (Chia et al., 2021; Fahrenfeld et al., 2019; Frei et al., 2019; Lwanga et al., 2017; McGechan, 2002; Viaroli et al., 2022; Wanner, 2021). MFs origin and transport mechanisms could be very similar to MP ones, however, these studies are at the early stage.

Throughout history, humans have explored every place on the surface of the planet, even the most remote and inaccessible, also with the help of different technologies capable of exploring these environments for them. The same does not apply to the subterranean world, in which passages still completely unknown, since there are no tools and technologies for these kinds of explorations. At the moment, the only way to increase knowledge about these environments is to explore them by yourself, therefore, the underground world remains the last frontier of terrestrial exploration. Evaluating MPs and natural and regenerated MFs occurrence in unexplored subterranean environments provide important insights for the management and conservation of karst areas, highlighting how indirect pollution can contaminate even places never explored by humans before.

In this study we investigated for the first time sediment samples from five unexplored caves of the Abruzzo Region, Italy. The aims of this study are: i) to examine, for the first time, the presence of MPs and natural and regenerated MFs in unexplored caves; if present, ii) to discuss AMP abundance and characteristics, potential risks and ecological effects that this kind of pollution could lead to karst environments, even indirectly. This work wants to be the first assessment of AMP pollution in unexplored environments, encouraging more researchers and speleogists to sample also during explorations, despite all the difficulties of progression in unknown environments, helping environmental studies on pollution, with the aims of promoting appropriate conservation measures for karst areas.

2. Materials and method

2.1. Study area

Examined caves are located in the village of San Vito, in the Valle Castellana municipality, Abruzzo Region, Italy (Fig. 1). Valle Castellana is part of the Gran Sasso and Monti della Laga National Park. The valley ranges from medium low altitudes (400 m) to high peaks that exceed 2400 m a.s.l. San Vito village is located at about 684 m a.s.l. The soil is predominantly of marly-sandstone, however, travertine is present in some areas, in which caves are present. The area is characterized by a mountain climate, with snowy winters and mild summers. The diversity of altitude, the abundance of water, and the variety of microclimates present favored the expansion of forests and dense woodlands in the past.

2.2. Field sampling

Five unexplored caves were selected for this study (Fig. 1E, F). For San Vito caves 2, 5, 6, 8, three sampling points at different distance from the entrance were defined, one for San Vito cave 4 (Table 1), based on the environmental characteristics of the caves. Sampling points were called from 1 to 3 starting from the entrance to the deepest areas of the caves (Fig. 1F). Monitoring was conducted in September 2022.

San Vito 2 cave (Fig. 1F) has a predominantly horizontal development, with an entrance of about 4.5 m characterized by the presence of a dry wall that partially protects the entrance. The entrance opens after a small descent of about 2 m, characterized by the foliage of the beech trees. The first sampling point (SV2-1) was located in the entrance hall, the second (SV2-2) after a narrow passage, under a small rock jump, and the last (SV2-3) at the end of the cave, at the foot of an ascent that connects San Vito 2 cave with the nearby San Vito 3 cave. Bones of small animals were found inside the cave.

San Vito 4 cave (Fig. 1F) has a semi-vertical development with a pit-entrance of about 3 m. The first room was unfortunately invaded by garbage. The only sampling point (SV4-1) was located at the end of a second room, distant about 10 m from the entrance.

San Vito 5 cave (Fig. 1F) has a predominantly horizontal development of about 22 m with a slightly sloping entrance of about 2 m. Although the presence of the provincial road (SP) San Giacomo-San Vito at about 10 m from the cave entrance, the entrance is not immediately visible, and is about 1.8 m wide by 1.5 m high. The cave develops along a fracture, with collapsed boulders. The first sampling point (SV5-1) was located after the slide at the entrance, rich in foliage and earth falling from the surface. In this area, a cave salamander and some arthropods were observed. The second sampling point (SV5-2) was located in the central part of the cave, and the third (SV5-3) at the end. The end of this cave is located under the SP. During the sampling, the soil sampled was barely wet, such as all the caves.

San Vito 6 cave (Fig. 1F) has a predominantly horizontal development of about 16 m with a slightly sloping entrance of about 5 m high, at the end of which was located the first sampling point (SV6-1). The entrance is located near the SP San Giacomo - San Vito. In the middle of the cave a pit of about 4 m is present (Sampling point SV6-2). The last sampling point was located at the end of the cave (SV6-3).

San Vito 8 cave (Fig. 1F) is located in a vegetated zone rich in ferns, mosses and other plants typical of highly humid areas, in an ex-travertine quarry, closed about 40 years ago. The cave entrance is about 4 m high and 1.7 m wide. The cave has a vertical development, characterized by about four wells. Sampling were done immediately under the first well (SV8-1), the third well (SV8-2), and at the end of the cave (SV8-3), characterized by the presence of mud, but without flowing water during the sampling period in September.

Caves and underground environments are typically extreme, which makes it challenging to gather large sample volumes. Unexplored caves, in particular, present logistical difficulties due to the unknown cave morphology and speleological challenges. The limited availability of sediment for analysis, coupled with the complexities of sample collection and transport, can create significant hurdles. Nevertheless, subterranean environments remain understudied, and unexplored caves have not yet been sampled. As a result, any sampling and subsequent analysis are crucial for advancing knowledge about these ecosystems' health and the indirect effects of human activities. Following the precautionary principle and evaluating the environmental characteristics of the studied cave, the amount of collected samples was limited to about 500 g.

Sediment bulk samples (Hidalgo-Ruz et al., 2012) were collected by the Speleo Club Teramo cavers during the cave explorations. Before sampling, speleologists were trained on methodology and sampling through training courses and the drafting of a protocol. Samples were collected with a metal spoon using nitrile gloves, and put inside boiled and pre-cleaned glass jars. The metal spoon was cleaned every time with ethanol. Samples were named with SV (San Vito), the number of the cave (2, 4, 5, 6, 8) and the number of the sampling point (from 1 to 3). The jars were limited and coated with anti-impact material to transport them safely in the speleological bags. For each sampling area, about 400–600 g of shallow sediments (upper 5 cm) were collected according to the availability.

Samples were stored in the fridge at 6 °C until analysis in the laboratory.

2.3. Laboratory analysis

2.3.1. Contamination control

Whenever possible, plastic laboratory equipment was substituted with glass and metal alternatives. Throughout all lab procedures, researchers wore nitrile gloves and white cotton lab coats. To prevent AMPs contamination, all open containers, laboratory glassware, and equipment were covered with aluminum foil during all analyses. All work surfaces and laboratory materials were thoroughly cleaned with ethanol and MilliQ water. All analyses were conducted within a fume hood.

Blank controls were performed using 30 % H_2O_2 (Merck), absolute

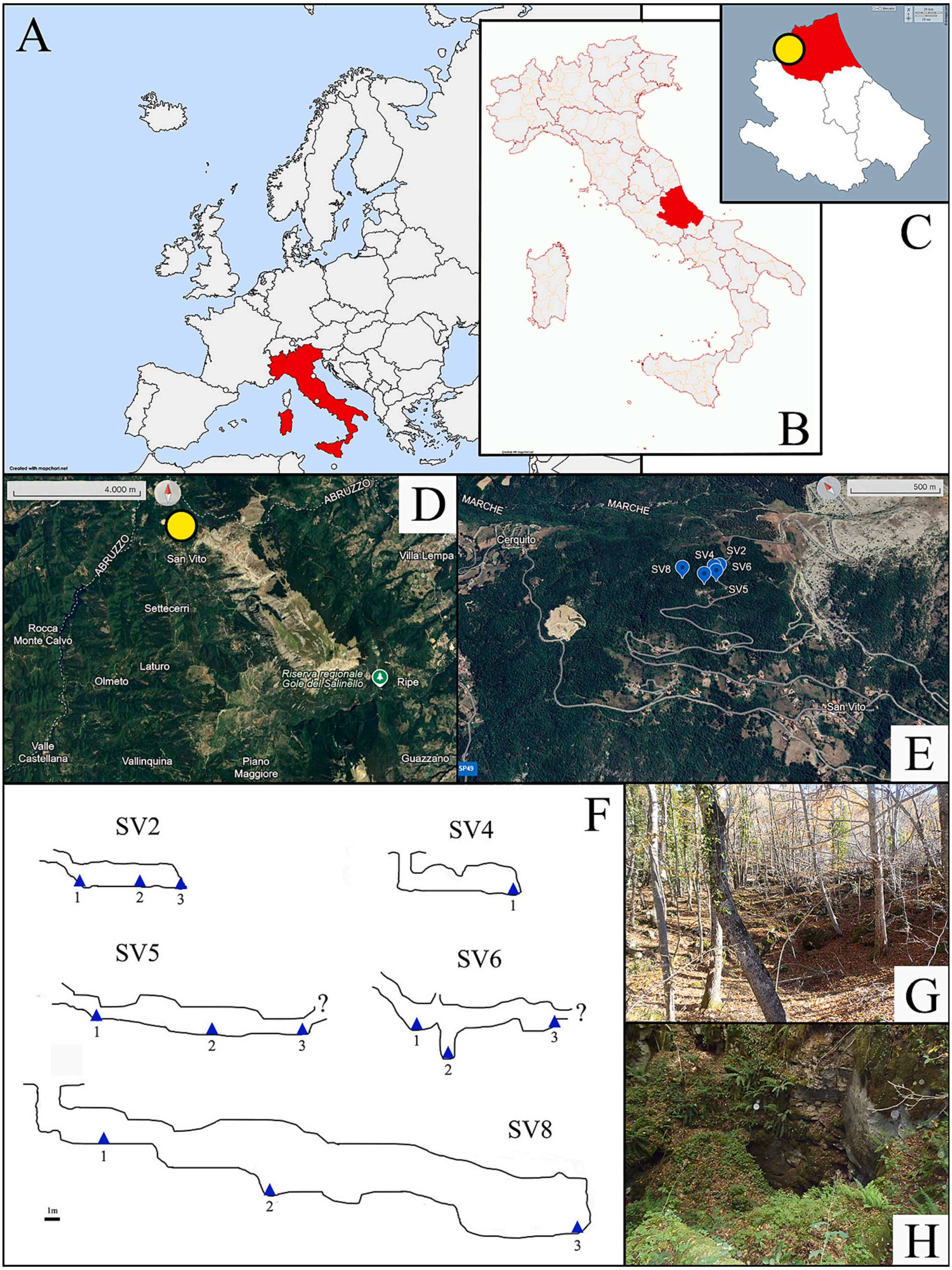

Fig. 1. Study area, caves and sampling points. A, B: Study area (maps from mapchart.net/italy, modified and mapchart.net/italy, modified); C: Abruzzo Region, Italy, with yellow point for the studied area (Map from https://d-maps.com/carte.php?num_car=21530&lang=it, modified); D: Detailed images of the area with yellow point for the studied area (map from https://earth.google.com/, Imagery ©2009 Google, Airbus, GeoBasis-DE/BKG (©2009), modified [access 2024-11-11]); E: Detailed images of the area with blue placemark for the monitored caves (Map from Google, Airbus (©2009), modified [access 2024-11-15]); F: Cave surveys (sections) with sampling points (blue triangles); G, H: photos from the monitored area near the caves (photos by E. Licocci).

Table 1
Information regarding the monitored caves and their respective sampling points.

Cave	Horizontal development [m]	Development	Sampling points	Sampling point distance from the entrance [m]
SV2	10	Horizontal	SV2-1	2.5
			SV2-2	7
			SV2-3	10
SV4	10	Semi-vertical	SV4-1	10
SV5	22	Horizontal	SV5-1	3
			SV5-2	13
			SV5-3	20
SV6	16	Horizontal	SV6-1	3
			SV6-2	10
			SV6-3	16
SV8	45	Vertical	SV8-1	7
			SV8-2	20
			SV8-3	45

ethanol (VWR Chemicals), MilliQ water, and a NaCl solution (Carlo Erba NaCl + MilliQ water) to assess potential AMPs contamination from the chemicals and water used. The blank correction method applied in this study assesses the results of unknown samples by subtracting the blank contribution (Shruti and Kutralam-Muniasamy, 2023 and references therein). The pollutants detected in the tested products were summed and then subtracted from the total amount found in the samples. All chemicals and MilliQ water were analyzed using the same method applied to the sediment samples. This method is common and easy to use, however, it only allows for the correction of data about abundances, without the ability to distinguish between particles related to contamination and those originally present in the sample. Consequently, data in this study about AMPs characteristics cannot be corrected.

2.3.2. *Sediment analysis*

Sediment samples were analyzed following the procedures outlined by Balestra et al. (2024a). The collected sediments were set in a tank of aluminum, covered with aluminum foil, and dried in an oven at 40°C until reaching a constant weight. Afterward, the dried sediments were weighed and subjected to organic matter removal (OMR) using a 1:1 30 % H_2O_2 solution. Finally, sediments were left to react for seven days under natural conditions, then dried again at 40 °C to constant weight.

Depending on the amount of remaining dry sediment in each sample, three sub-samples of 15 g each were selected using the coning and quartering method. These sub-samples were transferred into glass beakers containing 150 mL of NaCl solution (200 g NaCl/0.6 L, ρ = 1.2) and stirred for 2 min using a magnetic stirrer. Then, sub-samples were left to settle for 24 h to allow the heaviest material to deposit. The supernatant was carefully extracted using a glass pipette (approximately 50 mL) and filtered through a 0.2-μm pore size ANODISC 47 microfiber filter (Cytiva, Ø 47 mm) using a glass vacuum pump. Filters were then placed in glass petri dishes, covered with aluminum foil, and dried in an oven at 40 °C until completely dry.

2.4. *Microplastic identification and characterization*

A combination of microscopic and spectroscopic techniques was used in this work.

Different materials are produced using fluorescent additives (Qiu et al., 2015), such as plastics and textile fibres, therefore, many of them can be simply detected under an ultraviolet (UV) light (e.g. Balestra and Bellopede, 2022; Balestra et al., 2024a; Balestra et al., 2024b; Balestra et al., 2023; Ehlers et al., 2020; Giardino et al., 2023; Klein and Fischer, 2019; Qiu et al., 2015). However, not all plastics and fibres contain fluorescent additives, especially dark ones (Balestra and Bellopede, 2022; Balestra et al., 2024a; Balestra et al., 2024b; Balestra et al., 2023), and different natural materials, organic and inorganic, are fluorescent under a UV light. Therefore, a preliminary screening analysis is necessary to understand the nature of observable fluorescent materials.

Initially, microparticles on filters were observed under a Leitz ORTHOLUX II POL-MK microscope equipped with a DeltaPix Invenio 12EIII 12 Mpx Camera. They were counted at 2.5× magnification, enlarged to 10× or higher magnifications for identification and characterization. This step was carried out in accordance with the stringent selection criteria reported in previous works (Crawford and Quinn, 2016; Hidalgo-Ruz et al., 2012; Noren, 2007). A further detailed comparison was made for the MF analysis, observing details showed in longitudinal and x-sectional microscopic images of natural, regenerated and synthetic fibres (e.g. Khan et al., 2017). Each filter was examined with and without a UV Alonefire SV10 365 nm flashlight, 5 W. Microparticles ranging from 5 to 0.1 mm were analyzed, as visual identification of smaller particles is less accurate compared to larger ones (European Commission, 2013; Hidalgo-Ruz et al., 2012; Song et al., 2015). Microparticles that could not be definitively identified as AMPs were excluded from the analysis. Observed MPs were characterized according to the Standardised size and colour sorting system (SCS) (Crawford and Quinn, 2016). MFs were categorized into synthetic (MPs), non-synthetic (natural and regenerated materials processed chemically), and unknown (N.D.) (degraded anthropogenic material that could not be clearly identified).

Spectroscopic analyses were typically performed on 1 % to 10 % of the detected AMPs to determine their chemical composition (International Organization for Standardization and European Committee for Standardization, 2020). In this study, 40 % of the microparticles found on the filters from each sampling area was analyzed using a micro-Fourier Transform Infrared Spectroscope (μFTIR), Shimadzu AIM-9000 microscope, coupled with a Shimadzu IRTracer-100 spectrophotometer. The analysis was performed in attenuated total reflection (ATR) mode with a germanium prism (Shimadzu ATR). The microparticles on the filters were analyzed within the spectral range of 4000 to 700 cm^{-1}, with 40 scans taken per sample. Atmospheric corrections were applied to the obtained spectra. The spectra were compared with the Shimadzu Lab Solution Library ATR Polymer 2, and a visual comparison of the characteristic bands was performed to match the reference spectrum. Only spectra with a match degree ≥70 % were accepted.

3. Results

3.1. *Abundance and composition*

MPs were not found in the major part of the sediment sub-samples, however, in three sub-samples were present (Table 2). Caves SV4 and SV8 had no MPs, in the other monitored caves MPs were present only in one sampling point, and in relatively small amounts (2–4 items for 15 g of sediments) (Table 2).

In addition to MPs, taking into account also natural and regenerated MFs of anthropogenic origin, all monitored caves were polluted, with more contaminated sampling site (Fig. 2, Tables 2, and Supplementary Table 1). Considering that examined caves were unexplored, the quantities of AMPs found in the sampling points were considerable, with an estimate ranging from 0 to 2311.1 items/kg (Fig. 2 and Supplementary Table 1).

Taking into account the average amounts of microparticles found for each cave, SV4 was the most polluted cave, and SV8 was the least one (Table 3).

Microscopic analysis showed that 97.1 % of AMPs were natural and regenerated materials, 2.2 % synthetics, and 0.7 % not clearly distinguishable (Figs. 3A, 4). Of the natural and regenerated MFs, 34.1 % were cotton, ranging from 21.5 % to 58.1 % in caves SV4 and SV2 respectively. All other materials were cellulosic, except for one woolen MF found in cave SV5.

μFTIR-ATR analysis were useful to confirm microscopic analysis. Unfortunately, AMPs found in the sediments showed a high degradation degree and were often contaminated by different matter, therefore,

Table 2
Anthropogenic microparticles (AMP) abundances for each sub-sample of sediment from unexplored caves, and estimates for one kilo of sediments. AMPs were divided into synthetic (microplastics) and non-synthetic (natural and regenerated microfibres).

Filter Water	Natural and Regenerated [items/15g]	Synthetic [items/15g]	N.D. [items/15g]	TOT [items/15g]	Natural and Regenerated [items/kg]	Synthetic [items/kg]	N.D. [items/kg]	TOT [items/kg]
SV2 1.1	0	0	0	0	0	0	0	0
SV2 1.2	0	0	0	0	0	0	0	0
SV2 1.3	0	0	2	2	0	0	133	133
SV2 2.1	0	0	0	0	0	0	0	0
SV2 2.2	52	3	0	55	3467	200	0	3667
SV2 2.3	0	0	0	0	0	0	0	0
SV2 3.1	0	0	0	0	0	0	0	0
SV2 3.2	0	0	0	0	0	0	0	0
SV2 3.3	0	0	0	0	0	0	0	0
SV4 1.1	0	0	0	0	0	0	0	0
SV4 1.2	0	0	0	0	0	0	0	0
SV4 1.3	54	0	0	54	3600	0	0	3600
SV5 1.1	0	0	0	0	0	0	0	0
SV5 1.2	0	0	0	0	0	0	0	0
SV5 1.3	0	0	0	0	0	0	0	0
SV5 2.1	0	0	0	0	0	0	0	0
SV5 2.2	0	0	0	0	0	0	0	0
SV5 2.3	100	4	0	104	6667	267	0	6933
SV5 3.1	6	0	0	6	400	0	0	400
SV5 3.2	0	0	0	0	0	0	0	0
SV5 3.3	49	0	0	49	3267	0	0	3267
SV6 1.1	0	2	0	2	0	133	0	133
SV6 1.2	0	0	0	0	0	0	0	0
SV6 1.3	19	0	0	19	1267	0	0	1267
SV6 2.1	41	0	0	41	2733	0	0	2733
SV6 2.2	0	0	0	0	0	0	0	0
SV6 2.3	0	0	0	0	0	0	0	0
SV6 3.1	0	0	0	0	0	0	0	0
SV6 3.2	0	0	0	0	0	0	0	0
SV6 3.3	0	0	0	0	0	0	0	0
SV8 1.1	0	0	0	0	0	0	0	0
SV8 1.2	0	0	0	0	0	0	0	0
SV8 1.3	0	0	0	0	0	0	0	0
SV8 2.1	0	0	0	0	0	0	0	0
SV8 2.2	0	0	0	0	0	0	0	0
SV8 2.3	0	0	0	0	0	0	0	0
SV8 3.1	0	0	0	0	0	0	0	0
SV8 3.2	34	0	0	34	2267	0	0	2267
SV8 3.3	0	0	0	0	0	0	0	0
TOT	355	9	2	366	23667	600	133	24400
%	97.0	2.5	0.5	100.0		163.9	36.4	6666.7

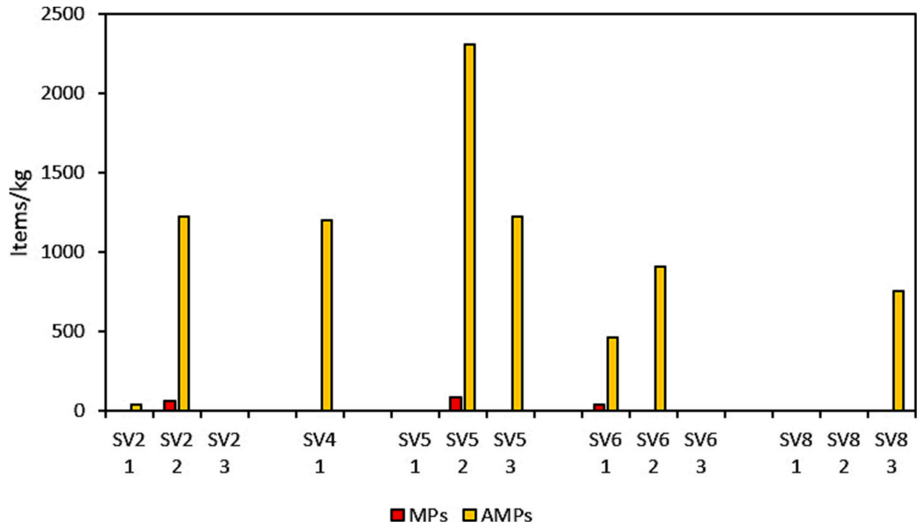

Fig. 2. Microplastic (MP) and anthropogenic microparticle (AMP) abundances for each sampling point.

Table 3
Anthropogenic microparticle (AMP) abundances for unexplored cave (averages). AMPs were divided into synthetic (microplastics) and non-synthetic (natural and regenerated microfibres).

Cave	Natural and regenerated [items/15 g]	Synthetic [items/ 15 g]	N.D. [items/ 15 g]	TOT [items/ 15 g]	Natural and regenerated [items/kg]	Synthetic [items/kg]	N.D. [items/ kg]	TOT [items/ kg]
SV1	5.8	0.3	0.2	6.3	385.2	22.2	14.8	422.2
SV4	18.0	0.0	0.0	18.0	1200.0	0.0	0.0	1200.0
SV5	17.2	0.4	0.0	17.7	1148.1	29.6	0.0	1177.8
SV6	6.7	0.2	0.0	6.9	444.4	14.8	0.0	459.3
SV8	3.8	0.0	0.0	3.8	251.9	0.0	0.0	251.9
MEAN	10.3	0.2	0.0	10.5	685.9	13.3	3.0	702.2

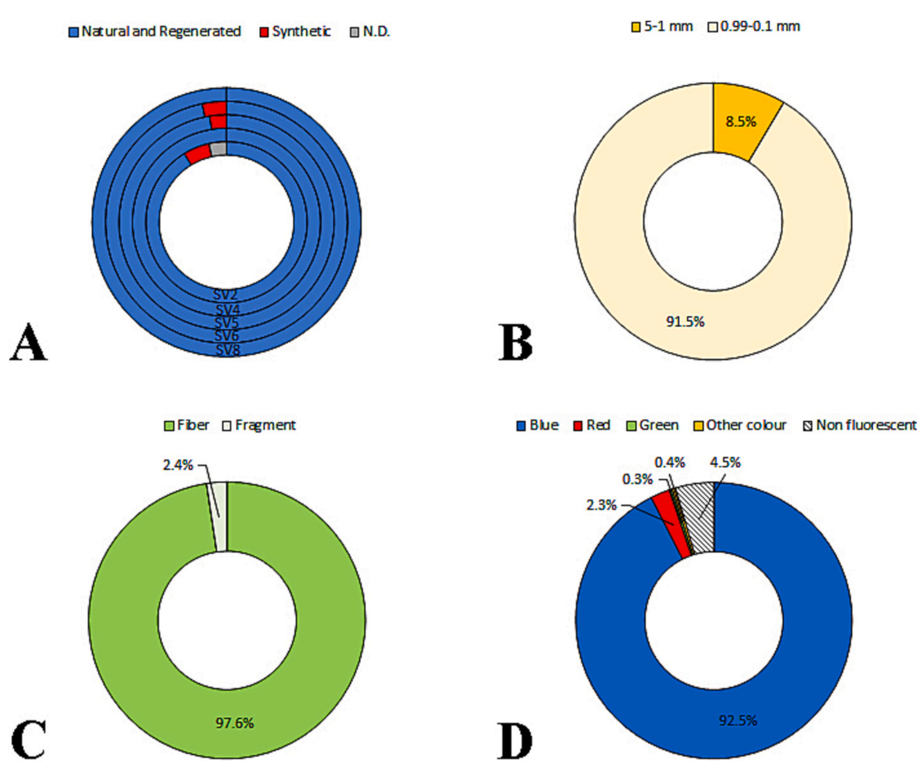

Fig. 3. Anthropogenic microparticle characteristics found in sediment samples from unexplored caves (mean of the collected data). A: microparticle composition; B: microparticle size; C: microparticle shape; D: microparticle colour of fluorescence.

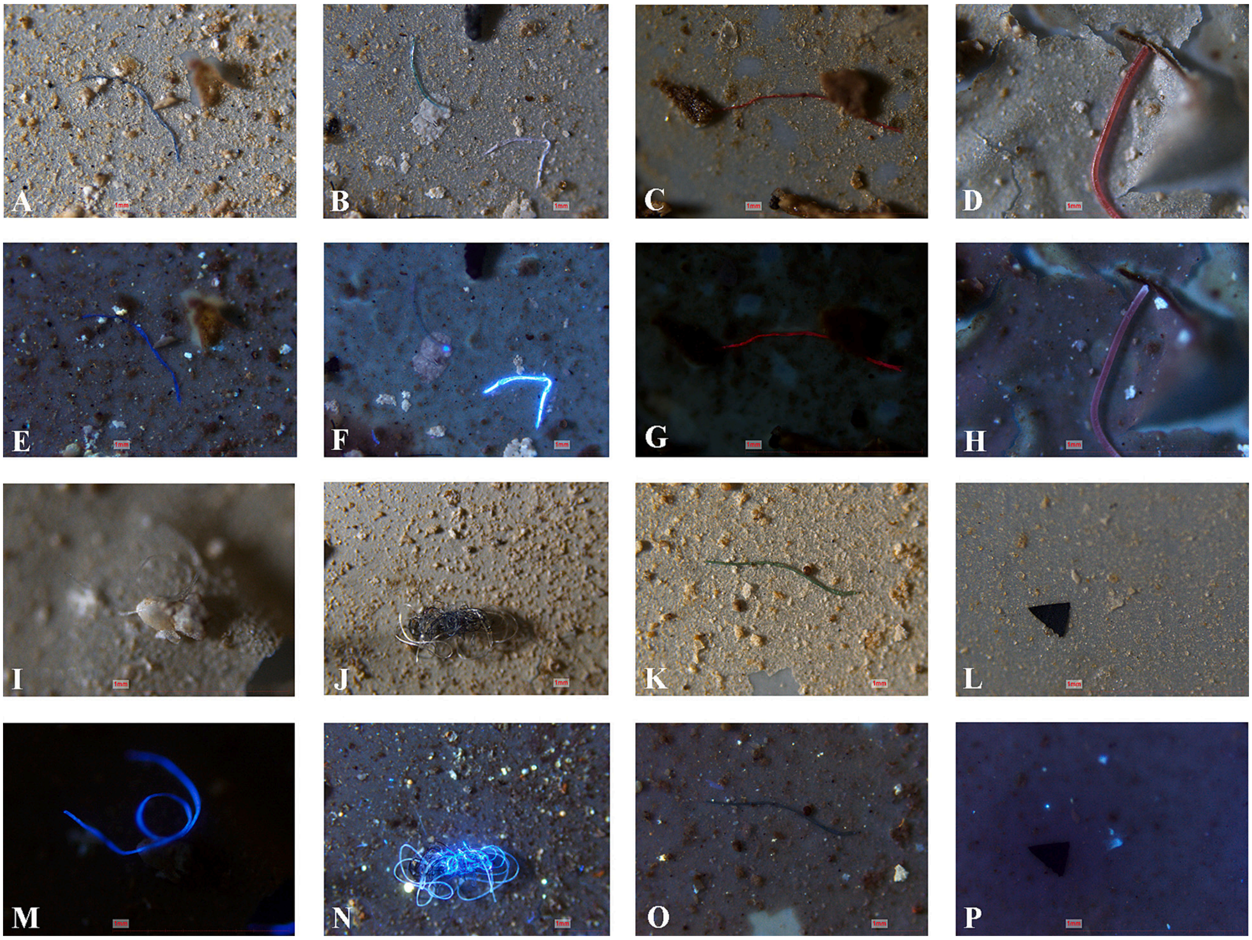

Fig. 4. Anthropogenic microparticles under microscope, with and without UV light. A, B, C, D, I, J, K, L without UV light; E, F, G, H, M, N, O, P with UV light. A–E: cotton blue fibre with blue fluorescence under UV light; B–F: light green synthetic fibre non fluorescent under UV light, and transparent cellulosic fibre with blue fluorescence under UV light; C–G: red cotton fibre with red fluorescence under UV light; D–H: red synthetic fibre with no fluorescence under UV light. I–M: transparent synthetic fibre with blue fluorescence under UV light; J–N: ball of fibres of different composition, colour, and fluorescence; K–O: green cotton fibre not fluorescent under UV light; black synthetic fragment not fluorescent under UV light.

spectroscopic analysis were not always possible, resulting in spectra with low matches. Analyzed AMPs (40 %) with good spectra confirmed that the major part of the particles were cellulosic MFs, transparent, with size <1 mm. Of the synthetic AMPs, analyses confirmed the presence of polyethylene terephthalate (PET), polyethylene chlorinated (CPE or PE-C) and silicon grease.

3.2. Size and shape

Size average percentages of analyzed microparticles were similar for all caves (Supplementary Table 2). AMPs <1 mm were the most abundant (91.5 %); big AMPs (5–1 mm) accounted for only 8.5 % (Fig. 3B). Three meso-MFs (5–25 mm) were also found: one in SV5 cave, sampling area 3, and two in SV8 cave, sampling areas 1 and 2.

Fibre-shape dominated all samples (97.6 %), followed by fragments (2.4 %) (Figs. 3C, 4 and Supplementary Table 2).

3.3. Fluorescence and colour

The 95.4 % of the analyzed AMPs were fluorescent under UV (Figs. 3D, 4, and Supplementary Table 2). Of the fluorescent particles, the major part had blue fluorescence (92.5 %) (Figs. 3D, 4, 5A and Supplementary Table 2). The non-fluorescent AMP abundance percentages in sediments were quite similar for each sampling area (average of 4.5 %), with the exception of SV2 cave, which has even higher values (5.8 %) (Fig. 5A and Supplementary Table 2).

Of the fluorescent AMPs found 91.8 % were transparent, followed by red (2.4 %), and blue (1.5 %) ones; particles with other colours had percentages between 0.3 and 1.0 % (Figs. 4, 5B). Non-fluorescent AMPs were mainly black (58.6 %), transparent (9.0 %), brown (8.4 %), and grey (7.2 %); particles with other colours had percentages between 1.3 and 6.2 % (Figs. 4, 5C). However, the percentages vary greatly from cave to cave, and also between different sampling sites within the same cave.

4. Discussion

4.1. The dark continent is polluted by anthropogenic microparticles

MPs were found only in the sediments of three sampling point (SV2-2, SV5-2 and SV6-1) with small amount: Cave SV4 and SV8 had no MPs (Table 2). However, taking into the account also natural and regenerated MFs of anthropogenic origin, all monitored caves resulted polluted, and with more contaminated sampling sites (Fig. 2 and Table 2). Considering that examined caves were unexplored, the quantities of AMPs found in the sampling points were comparable to values found in known caves (e.g. Balestra et al., 2024a), with an estimate ranging from 0 to 2311.1 items/kg (Fig. 2 and Supplementary Table 1). The most polluted cave resulted SV4, the least one SV8 (Table 3). Cave SV4 had a lot of garbage inside, in the first room, therefore, the high AMPs amount could be directly linked to the litter inside the cave. Cave SV8 is located

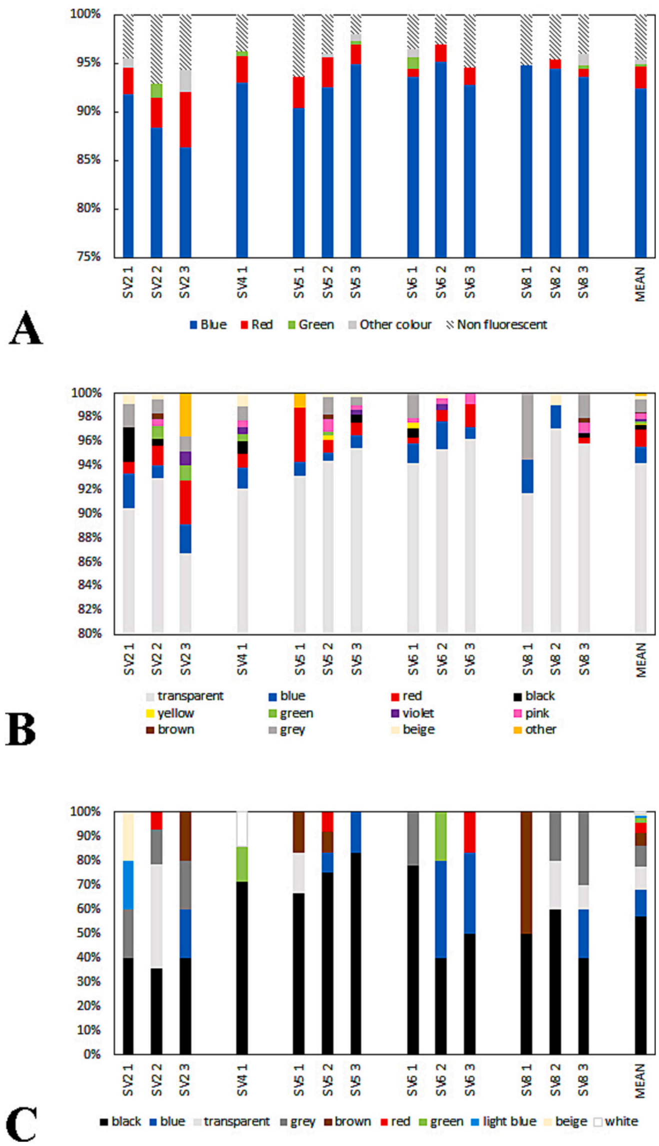

Fig. 5. Anthropogenic microparticle (AMP) fluorescence and colour for sampling site.. A: Fluorescence; B: Colour of fluorescent AMPs; C: Colour of non-fluorescent AMPs.

in a more wooded area respect to the other caves, and it is also the most distant from road and villages, suggesting that the proximity to human activities has a major influence on pollution in the caves.

Comparisons with other unexplored caves are not feasible at the moment, as this research is the first conducted in unexplored underground environments. Furthermore, making comparisons between studies of MPs and MFs is generally challenging, as differing methodologies and analytical approaches can influence the results. Variables such as the size of the microparticles considered, the sampling location, and environmental conditions may also lead to fluctuations in concentrations over time. Nevertheless, some assumptions can be made regarding the presence of MPs and MFs in karst subterranean environments (Table 4).

MPs found in the Abruzzo unexplored caves highlighted that MP abundance is lower than show caves sediments (Balestra and Bellopede, 2022; Balestra and Bellopede, 2023; Hasenmueller et al., 2023) and submerged sediments in the caves of the Italian Classical Karst area investigated so far (Balestra et al., 2024a; Balestra et al., 2024b) (Table 4). However, they resulted higher than those found in the examined Italian marine caves (Bergamin et al., 2024; Romano et al., 2023) and in the caves of the Slovenian Classical Karst (Valentić et al., 2022) (Table 4). Considering also MFs, the abundances of AMPs are higher, more similar to that found in the speleological area of Bossea cave, Italy (Balestra and Bellopede, 2022), or in Cliff cave, a US show cave with limited visitor access (Baraza and Hasenmueller, 2023; Hasenmueller et al., 2023) (Table 4). It must be specifically takes account that the size range of detected microparticles in these works is different, as well as used methodologies, and examined caves have totally different environmental (submerged sediments in fresh and sea water, sediments after a flood, sediments away from water), climatological, and anthropogenic sources (tourism or not, presence of roads, railway and villages near the caves or wild areas) conditions. Moreover, someone include anthropogenic MFs in the abundances, others separate MPs from MFs.

Microscopic analysis showed that 97.1 % of AMPs were natural and regenerated materials, 2.2 % synthetics, and 0.7 % not clearly distinguish. Of the natural and regenerated MFs, 34.1 % resulted cotton, with averages for cave ranging from 21.5 % in cave SV4 to 58.1 % in cave SV2. All other materials were cellulosic, except a wool MFs found in cave SV5. μFTIR-ATR analysis generally confirmed the microscopic ones, highlighting a prevalence of transparent cellulosic fibres, but, unfortunately, spectra were not good, due to the AMPs degradation degree, and especially by different materials contamination on microparticles and filters. Analysis on synthetic AMPs showed the presence of PET, PE-C, and silicon grease. PET is commonly used in the textile industry, while PE-C is used in cable and rubber industries. Silicone grease is not strictly considered plastic, but rather a substance derived from silicone polymers, which are a type of synthetic material, therefore, someone include this particles in MPs research. MP typology comparisons with other karst areas are possible only with few works (An et al., 2022; Balestra and Bellopede, 2023; Balestra et al., 2024a; Balestra et al., 2024b; Balestra et al., 2023; Romano et al., 2023; Valentić et al., 2022), where polyesters, polyamide (PA), and copolymers have been found frequently; PE-C and silicon grease were less reported. Inside the Cliff cave, US, 31 % of particles was cellulosic and 29 % plastic (Hasenmueller et al., 2023), values quite different from our study. However, our values are similar to that found in other environments, for example in the oceanic surface waters, in which the major part of the analyzed MFs were natural (91.8 %), of which 79.5 % cellulosic and 50 % of all fibres cotton; only 8.2 % fibres were synthetic, of which 6.2 % polyester (Suaria et al., 2020a). In natural environments, different research reported that non-synthetic MFs were more abundant that synthetic ones, and especially cellulosic fibres, principally cotton (Athey and Erdle, 2022 and references therein). Given that approximately 67 % of textile production worldwide in 2023 was synthetic (Textile Exchange, 2024) there is a divergence between the MF composition identified in natural environments and the global production of synthetic textiles, requiring more in-depth research. These data could confirm the presence and accumulation of anthropogenic cellulosic fibres in the environment over time. However, 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.

Another interesting discussion topic regards the position of the AMPs respect to the cave entrance and morphology. In caves SV2, SV5 and SV6, the major part of the AMPs was found in the middle of the cave. This could be linked to the morphology of these caves, which have a predominantly horizontal development, white large entrances or slightly sloping ones, favoring the displacement of materials. Moreover, cave SV6 has a pit in the middle of the cave in which materials can accumulate. In SV4, SV5 and SV8 caves the inner part resulted polluted, while in SV2 and SV6 caves, AMPs were not found. Cave SV4 has a semi-vertical development, and the first area was polluted by litter, therefore, AMPs could be transported into the inner part by gravity, water and air circulation. Cave SV8 is the only vertical cave, characterized by a series

Table 4
Microplastic and microfibre concentration found in cave sediments in previous works.

Cave	Sampling information	Considered size	MP/MF concentration	References
Bossea cave Piedmont, Italy Show cave area	Surface sediments (5 cm) along the show cave path	5–0.1 mm	From 2500 to 8700 items/kg	Balestra and Bellopede (2022)
Bossea cave Piedmont, Italy	Surface sediments (5 cm) in a speleological area of the cave	5–0.1 mm	1600 items/kg	Balestra and Bellopede (2022)
Toirano caves Liguria, Italy	Surface sediments (5 cm) along the show cave path	5–0.1 mm	3823 items/kg (average)	Balestra and Bellopede (2023)
Toirano caves Liguria, Italy	Surface sediments (5 cm) in a speleological area of the cave	5–0.1 mm	3186 items/kg	Balestra and Bellopede (2023)
Borgio Verezzi cave Liguria, Italy	Surface sediments (5 cm) along the show cave path	5–0.1 mm	4695 items/kg (average)	Balestra and Bellopede (2023)
Borgio Verezzi cave Liguria, Italy	Surface sediments (5 cm) in a speleological area of the cave	5–0.1 mm	2933 items/kg	Balestra and Bellopede (2023)
214 Cave Friuli-Venezia-Giulia, Italy	Speleological caves, submerged sediments	5–0.1 mm	1267 MPs/kg	Balestra et al. (2024b)
214 Cave Friuli-Venezia-Giulia, Italy	Speleological caves, submerged sediments	5–0.1 mm	8982 MFs/kg	Balestra et al. (2024a)
Trebiciano cave Friuli-Venezia-Giulia, Italy	Speleological caves, submerged sediments	5–0.1 mm	2178 MPs/kg	Balestra et al. (2024b)
Trebiciano cave Friuli-Venezia-Giulia, Italy	Speleological caves, submerged sediments	5–0.1 mm	2649 MFs/kg	Balestra et al. (2024a)
Mariano Well Friuli-Venezia-Giulia, Italy	Speleological caves, submerged sediments	5–0.1 mm	911 MPs/kg	Balestra et al. (2024b)
Mariano Well Friuli-Venezia-Giulia, Italy	Speleological caves, submerged sediments	5–0.1 mm	2938 MFs/kg	Balestra et al. (2024a)
Bue Marino cave, Sardinia, Italy	Surface sediments (2 cm) in a speleological area of the marine part of the cave	25 mm-8 μm	From 10 to 27 items/kg	Romano et al. (2023)
Argentarola cave, Tuscany, Italy	Surface sediments (2 cm) of the marine caves	5–0.1 mm	From 5 to 12 MPs/kg	Bergamin et al. (2024)
Planina cave, Slovenia	Sediments	5–1 mm	0 MPs/kg	Valentić et al. (2022)
Postojna cave, Slovenia	Sediments	5–1 mm	0 MPs/kg	Valentić et al. (2022)
Škocjan caves system, Slovenia	Sediments	5–1 mm	60,000 MPs/m^3 (circa 35 al kg)	Valentić et al. (2022)
Kačna cave, Slovenia	Sediments	5–1 mm	6667 MPs/m^3 (circa 3.7 al kg)	Valentić et al. (2022)
Jama 1 v Kanjaducah, Slovenia	Sediments	5–1 mm	0 MPs/kg	Valentić et al. (2022)
Cliff cave, Missouri, US	Sediments during a flood	5 mm–? μm	8423 ± 166 particle/kg	Hasenmueller et al. (2023)

of wells, which favor the falling of matter and pollutants towards the inner areas. Moreover, the presence of mud at the end of this cave, highlight also the presence of water in some periods, which could transport and accumulate materials in the inner parts.

According to previous studies (Balestra and Bellopede, 2022; Balestra and Bellopede, 2023; Balestra et al., 2024a; Balestra et al., 2024b; Hasenmueller et al., 2023; Valentić et al., 2022), the major part of AMPs in the examined karst environments were fibres. Fragments found in our study had not a relevant percentage respect other previously examined caves (e.g. Balestra and Bellopede, 2023).

Being unexplored caves, pollution in the karst subterranean environments comes from the surface anthropogenic activities, atmospheric depositions, and infiltrations through fractures and soil. The high presence of fibres in the unexplored caves suggests that pollution in this area could be linked mainly to textiles deterioration. MFs could be transported in this area by atmospheric deposition, reaching the caves by gravity, water and/or air transport, or could be linked to geo-textiles deterioration from the roads built near some caves, such as SV5. Fragments may have originated from the breakdown of waste in the environment or from nearby human activities. Some of the caves monitored are located near the provincial road, so the presence of 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). Our values were similar to that found inside the Cliff cave, US, in which 91 % were fibres (Hasenmueller et al., 2023).

As reported in recent research (Balestra and Bellopede, 2022; Balestra and Bellopede, 2023; Balestra et al., 2024b; Balestra et al., 2023), most of the AMPs found were fluorescent under UV light (95.5 %). These percentage was higher than those found in the sediments of Bossea (88 %), Toirano (60 %), and Borgio Verezzi (75 %) caves, Italy, but similar to that found in the Italian sector of the Classical Karst, considering also MF pollution (mean of 93 %) (Balestra et al., 2024a). These data indicate that using UV light to identify AMPs is an effective method, though it is not enough for a thorough particle analysis. The fluorescence of AMPs could also provide valuable information about their consumption by organisms. For example, fluorescent AMPs were found in stygofauna sampled from three groundwater bodies in Italian karst regions (Sforzi et al., 2024). The health of ecosystems is significantly impacted by the accumulation of AMPs, as some organisms mistakenly ingest them, confusing them for food sources (Devereux et al., 2021; Gomiero et al., 2018; Pukos et al., 2023; Sforzi et al., 2024). AMPs can lead to a range of harmful effects on organisms, including pathological changes, disruptions in digestive processes, and reproductive deficits (Assas et al., 2020). Entering in the food chains, AMPs could have implications that extend to higher trophic levels, leading to cascading ecological effects (Zhang et al., 2023). In subterranean karst environments, AMPs may also affect the boundaries of these ecosystems, including vulnerable ecotones (Balestra et al., 2024b). As shown in previous research on MPs in biota of different environments, the colour of AMPs can give useful information on consumption by organisms (Carpenter et al., 1972; Jahan et al., 2019; Lusher et al., 2013; Romeo et al., 2015; Ugwu et al., 2021). This makes this step of analysis important for ecological and environmental research. Regarding AMPs ingestion by fauna, in marine biota, most of MPs particles found were blue, white, black, and transparent (Ugwu et al., 2021). Some studies found that black plastics were the most commonly ingested by marine animals (Jahan et al., 2019; Lusher et al., 2013), other ones showed that transparent and white MPs are the most found in the stomach of some top sea predators (Romeo et al., 2015). Instead, mid-tone (green, red, blue, etc.) AMPs were found in terrestrial birds (Zhao et al., 2016). In karst areas, blue 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 this study, transparent particles were found in the highest amounts in the sediments of all examined caves, as in Cliff cave, US, in the Italian show caves, or in the caves monitored in the Classical Karst, Italy (Balestra and Bellopede, 2022; Balestra and Bellopede, 2023; Balestra et al., 2024a; Balestra et al., 2024b; Hasenmueller et al., 2023). Different other colours were present in the examined unexplored caves, especially blue and red. Many studies are present on aquatic organisms, but they are less common in terrestrial ones, and studies on hypogeal animals are still very poor. Future research could further explore whether hypogeal organisms, particularly troglobionts, mistakenly consume AMPs as food sources. AMP fluorescence and colour can be significant for their ingestion by epigeal fauna or organisms less adapted to the subterranean environment. Scientific observations were made even regarding the relationship between MP colour and the chemical pollutants that can bind to them, leading to contamination (e.g. Frias et al., 2010; Karapanagioti et al., 2011). For instance, high levels of pollutants were found on yellow and black particles in marine environments (Frias et al., 2010; Karapanagioti et al., 2011). In our caves, black particles were the most common between the non-fluorescent AMPs, and present in all sampling points, however, not-fluorescent AMPS were only 4.5 % (Fig. 4B, C). Yellow particles were sporadic only in two sampling points (Fig. 4B).

AMP pollution in karst areas threatens both water quality and biodiversity, making it a critical concern for conservation. Monitoring AMPs in subterranean environments results essential for evaluating their health. Monitoring of AMP pollution in unexplored environments is a key element in understanding how and when human activity also indirectly affects uncontaminated environment, helping environmental studies to better understand sources and transport on pollutant, with the aims of promoting appropriate conservation measures for karst areas. Working in extreme and dark environments is not easy, and the difficulties of progression in unknown environments can be really hard. Large volumes of samples are not recommended in fragile ecosystems, and collecting samples and transporting them can be challenging, due to narrow and rope passages, wells, and unstable areas. However, underground environments are rarely studied, and the dark continent remains the last frontier in terrestrial explorations, consequently, any new information is crucial for a better understanding of the ecosystems themselves, and possible threats. Greater effort in sampling underground environments is essential, and even during exploration, collecting samples would enhance our knowledge on these very important systems. Effective protection and conservation strategies must take into account the ecological connections between surface and subterranean environments.

Promoting political and social changes is crucial to support genuine plastic reduction initiatives and encourage manufacturers to adopt more sustainable, eco-friendly materials for all products. Education at all levels, grounded in the principles of environmental sustainability, will be key to the management and conservation of water resources, protected habitats, and species.

4.2. Methodological considerations

Research on AMPs is challenging for several reasons, including external contamination, the selection of appropriate methodologies, and issues related to sampling and analysis.

Although the sampling in caves was carried out according to the chosen methodology, it should be considered that contamination, albeit limited, may be occurred during sampling, because of the material of the speleological clothes. However, speleologists wore speleological suits made of polyamide (PA) and cordura, a type of rayon, red and black. Helmets have outer shell in acrylonitrile butadiene styrene (ABS) or polycarbonate (PC) and inner foam in expanded polystyrene (EPS) or expanded polypropylene (EPP). The harnesses are composed of PA and polyvinyl chloride (PVC). The speleological bags are made of PVC or thermoplastic polyurethane (TPU), and gloves are generally made of PE, polypropylene (PP), PA with nitrile coatings, a synthetic rubber. The ropes are made of PA or polyester (PE). None of these materials were found in the analyzed samples.

High levels of AMPs were detected in the chemicals and solutions tested in this research, highlighting the importance of pre-cleaning and filtering all products before laboratory analysis. Increased awareness is needed regarding the extent to which these materials can contaminate samples, both for scientists and producers. Immediate action is necessary to improve the quality of their products and reduce potential pollution.

OMR is a necessary step before density separation, however, certain chemicals used in the process may lead to the partial or complete degradation of non-synthetic AMPs (Athey and Erdle, 2022; Treilles et al., 2020). H_2O_2 digestion is the most commonly used method for MP and MF analysis (Athey and Erdle, 2022), but it can alter the mechanical properties and IR spectra of the samples, increasing the fragility of certain types of MFs, especially cellulosic. The brittleness could potentially lead to MF fragmentation, causing counting errors and potential overestimation (Treilles et al., 2020).

The density separation method used for sediment samples in this research may limit our ability to capture anthropogenic materials with densities >1.2 kg/L. The specific density of different cellulosic materials is higher than that of several plastics, such as polyester, polypropylene, and nylon/acrylics. However, the densities of materials in natural environments may differ from those of newly produced materials. Due to factors like increased porosity, degradation, biofouling, or organic matter adsorption, the density of these materials can either increase or decrease over time in natural environments (Kaiser et al., 2017). Indeed, we observed materials with reported densities >1.2 kg/L, consistent with findings from surface water systems (Horton et al., 2017). However, it is important to consider that some materials with higher densities may remain in the sediments due to their inability to be separated by our method.

Microscopy is one of the most commonly used methods for MP and MFs characterization, especially due to its cost-effectiveness. However, visual analysis cannot provide information on the chemical composition of the particles, is a hardworking process, and is not recommended alone for small particles (European Commission, 2013; Hidalgo-Ruz et al., 2012; Huang et al., 2023; Song et al., 2015). Upside, this method allows for a clearer observation of the surface morphology of AMPs, as well as important characteristics such as colour. A preliminary microscopic screening can be helpful in distinguishing between synthetic materials and natural or regenerated ones.

Spectroscopic analyses are valuable for determining the chemical composition of microparticles, helping to confirm or refute the nature of particles identified through microscopy. However, these methods are time-consuming too, require expensive equipment and specialized personnel. The surfaces of particles collected from natural environments are often coated with other materials, impurities, and/or contaminated with additional pollutants, making it challenging to match the obtained spectra with high confidence to spectra libraries (Song et al., 2015). The methodologies developed for detecting and characterizing MFs were initially designed for MPs, therefore, analysis on natural and regenerated materials could be challenging (Athey and Erdle, 2022), from OMR to microscopic and spectroscopic analysis. Natural polymers exhibit lower signal intensities compared to synthetic ones and are more susceptible to interference from dyes. Additionally, the FTIR spectra of natural and regenerated polymers are nearly identical (Comnea-Stancu et al., 2017), and the presence of dyes, oxidation, and microbial degradation can also alter the absorption bands of cellulose in the FTIR spectra (Li et al., 2010; Remy et al., 2015; Zambrano et al., 2019). Therefore, distinguishing between them is extremely challenging. Misestimations in library matches could pose significant issues, which is why only high-quality matches (over 70 %) of spectra were used in this research.

A combination of multiple methods is the most effective approach for identifying AMPs in natural environments (Song et al., 2015), as detected in this study.

5. Conclusion

These results contribute to a better understanding of MP and MF pollution in natural environments, highlighting the potential risks to subterranean ecosystems.

This study is the first that documents the presence of MPs and MFs in unexplored subterranean environments, demonstrating that human activities indirectly impact also the dark continent, the last frontier of terrestrial explorations. This work wants to be the first assessment of AMP pollution in unexplored environments, encouraging more researchers and speleogists to sampling also during explorations, despite all the difficulties of progression in unknown environments. However, little is done in these environments, consequently, any new information is crucial to better understand these ecosystems and possible threats, helping environmental studies on pollution, with the aims of promoting appropriate conservation measures for karst areas.

While the concentration of MPs found was really low or absent in the monitored caves, the presence of MFs in sediments was higher and more variable. Most of the particles were transparent and fluorescent under UV light, while non-fluorescent particles were predominantly black or dark. Fibre-shaped particles were the most common, suggesting that textiles are a primary source of pollution in this area, probably linked to nearby human activities and roads, or atmospheric deposition, providing valuable insights for future research.

Surface and underground environments in karst areas are closely interconnected, so greater efforts should be made to implement more comprehensive protection measures. Expanding analyses to include a wider range of surface and subterranean habitats is essential. Future research is needed to better understand the sources and transport of MPs and MFs in karst areas, as well as their potential effects on ecosystems and organisms. Long-term monitoring will help clarify the impact of direct or indirect AMP pollution on karst habitats.

CRediT authorship contribution statement

Valentina Balestra: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rossana Bellopede:** Writing – review & editing, Resources, Project administration, Funding acquisition.

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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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.179328.

Data availability

Data will be made available on request.

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