Preliminary investigations of microplastic pollution in karst systems, from surface watercourses to cave waters

(Article begins on next page)

Preliminary investigations of microplastic pollution in karst systems, from surface watercourses to cave waters

Valentina Balestra[a,*], Bartolomeo Vigna[a], Sean De Costanzo[b], Rossana Bellopede[a]

[a] Department of Environment, Land and Infrastructure Engineering, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy, valentina.balestra@polito.it [0000-0002-8529-468X], bartolomeo.vigna@polito.it [0000-0003-2272-8740], rossana.bellopede@polito.it [0000-0002-9569-0678]

[b] Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy

* Corresponding author

Abstract

Microplastic pollution in different environments has increasingly been documented in detail in recent times, but it is still poorly studied in caves and karst aquifers. To deepen the knowledge of microplastic pollution, for karst environment protection and conservation purposes, we collected and investigated different water samples from a karst area of Italy, considering connected surface and cave waters. Microplastics were extracted from water samples by filtration and subjected to organic matter removal with 15% hydrogen peroxide solution. Microplastics on filters were counted and characterised (size, colour, shape) via visual identification under a microscope, with and without UV light, exploiting fluorescence given by fluorescent whitening additives (FWAs) contained in plastic materials. Finally, spectroscopic analyses were carried out on 10% of the microplastics observed on each filter. The concentration of microplastics in cave waters varied from 12 to 54 items/L, with a mean value of 28 items/L. In the surface water of a tributary, it was of 23 items/L, and in the downstream, it was 29 items/L. Fibres represented the most abundant shape (95.1%) in the karst system waters, and most microplastics (82.9%) were smaller than 1 mm. The majority of the microplastics were fluorescent under UV light (84.8%), and most fluorescent particles were transparent (46%). However, black microplastics (68%) were more common among the non-fluorescent ones. Polyethylene (51.7%) was the main type of microplastics found in the karst system waters. Our results show the presence of microplastics in karst systems and provide useful information for future research. Karst aquifers are open systems, subjected to possible contamination by surface pollutants. Microplastics in karst systems can be consumed by animals, damage ecosystems and contaminate water resources; surface karst areas and underground environments should therefore be monitored and protected, especially regarding the management of water resources. To further understand the sources and transport of microplastics within a karst system, analyses on a greater range of surface and subterranean waters throughout the world are required.

Keywords: microplastics, show caves, aquifers, water pollution, karst areas, groundwaters

1. Introduction

Microplastics (MPs) are defined as plastic particles smaller than 5 mm and can be produced intentionally (primary MPs), mainly for cosmetics or body care products, or via the degradation and/or fragmentation of plastic products due to physical, chemical and biological processes (Corami et al., 2020; Henry and Klepp, 2018; Prata et al., 2019). Microplastic contamination has been found in marine and terrestrial environments worldwide (e.g. Boyle and Örmeci, 2020; Sharma and Chatterjee, 2017; Wong et al., 2020) from populated areas (e.g. Jahan et al., 2019; Zhou et al., 2019) to remote ones (e.g. Ambrosini et al., 2019; Cabrera et al., 2020; Cincinelli et al., 2017; Van Cauwenberghe et al., 2013; Zhang et al., 2021) and can be extremely mobile. Microplastics are consumed by several organisms (Assas et al., 2020; Devereux et al., 2021; Henry and Klepp, 2018; Romeo et al., 2015; Wright et al., 2013), resulting in direct or indirect physical harm, and can be sources and vectors for other contaminants, such as persistent organic pollutants (POPs), heavy metals, pesticides and antibiotics (Li et al., 2018; Li et al., 2019; Rochman et al., 2013; Selvam et al., 2021; Zhou et al., 2019). Assessing MP presence and distribution in natural environments, using accurate methods for quantification, understanding their transport pathways and evaluating their impacts on organisms and habitats are necessary steps to be addressed for conservation purposes.

However, research on MPs is generally focused on the marine environment and surface ecosystems, and only few studies have examined MP pollution in caves and underground systems (e.g. Balestra and Bellopede, 2022b; Christman, 2019; Vardar and Vidal Rodriguez, 2021). Regarding freshwater resources, research is in its early stages. The potential contamination of groundwater is often only mentioned, and a limited number of studies focused on groundwater MP pollution (e.g. Khant and Kim, 2022; Mintenig et al., 2019; Panno et al., 2019; Samandra et al., 2022; Selvam et al., 2021; Viaroli et al., 2022). Moreover, MP studies suffer from methodological discrepancies, providing heterogeneous analysis results, and the invisibility of the subterranean karst system structure makes impedes the study of such systems, even if they preserve a precious asset such as groundwater.

Sources of MPs to groundwater include wastewater, litter, atmospheric deposition and soil and surface waters pollution. Atmospheric transport favours the distribution of MPs over long distances in relation to wind direction and intensity, rain/snow precipitation intensity and duration (Allen et al., 2019). In the hydrogeological cycle, atmospheric water contribution is the main aquifer recharge. The presence of MPs in precipitation was detected in cities and remote areas (Allen et al., 2019; Liu et al., 2019), highlighting the omnipresence of this contaminant. Therefore, MP contamination in soil is strongly related to precipitation, facilitating MP infiltration (Zhou et al., 2021). When MPs are deposited on the ground, some can be transported vertically into the subsoil, travelling over long distances throughout the rock fractures and accumulating in the groundwater system (Chia et al., 2021; Wanner, 2021). Previous studies highlighted the downward movement of microparticles through soil pores and cracks for smaller MPs (Fahrenfeld et al., 2019; McGechan, 2002; Viaroli et al., 2022), and vertical and horizontal migration in soil matrices could be also be related to bioturbation (Lwanga et al., 2017). The hyporheic zone, the zone between surface and groundwater, is an important accumulation zone for MPs of small dimensions (Frei et al., 2019). The hydrogeological setting of the basin and seasonal trends must be taken into account, as well as the flow regime of streams and riverbeds. Therefore, hydrogeological information and aquifer types are decisive in choosing the sampling approach and detection method.

Karst aquifers are groundwater environments usually made up of carbonate rock, constituting about 25% of the global drinking water sources (Panno et al., 2019). The hydrodynamic regime of these aquifers is affected by the geology and the rock cluster fracturing, the karstification degree and the local meteorological conditions (Braun, 1984; Chauve et al., 1990; Hottelet et al., 1993; Moindrot et al., 1988). Considering the drainage system, three basic conceptual aquifer models have been described: systems with dominant drainage (absent or reduced phreatic zone, high permeability), systems with interconnected drainage (extensive phreatic zone, high permeability) and systems with dispersive circulation (highly extensive phreatic zone, middle–low permeability) (Balestra et al., 2022a; Ford and Williams, 2013; Ford and Williams, 1989; Vigna, 2007; Vigna and Banzato, 2015; White, 1969). Various intermediate situations exist, which can be described using these models considering hydro-chemical data and spring hydrography (Banzato et al., 2011). Besides being important drinking water resources, karst ecosystems and waters are particular habitats for troglobitic and endemic species (e.g. Balestra et al., 2022b; Barzaghi et al., 2021), which may be vulnerable to pollution (Manenti et al., 2018; Sket, 1999), and karst caves have an exceptional scientific value and are a precious archive that must be protected from damage, allowing paleo-environmental and paleo-climatic reconstructions and preserving palaeontological and archaeological finds (Balestra et al., 2021; Cigna and Forti, 2013). The open nature of karst aquifers makes them vulnerable to contamination by surface pollutants, which can be transported through the rock fractures (White, 1988) or can directly access the karst systems through some caves, especially tourist ones, increasing the pollution risk (Balestra and Bellopede, 2022b; Balestra et al., 2021). Fractured and karst aquifers are subjected to greater pollution risk as MPs are prone to circulate throughout the discontinuities, even if mechanical dispersion may attenuate the contaminant concentration in some zones (Viaroli et al., 2022).

Currently, to the authors´ knowledge, there is only one study on MP pollution in waters of karst areas, monitoring springs and wells under low-flow conditions (Panno et al., 2019). Studies on cave waters, as well as MP pollution in surface and underground waters of the same karst area, are lacking. The vulnerability of groundwater resources to pollution, anthropogenic pressure and climate change, as well as their important role in natural ecosystems and as a fundamental drinking water reserve, require actions targeted at underground environment protection and sustainable resources management (Balestra et al., 2021; Re, 2019). Therefore, investigations are required for understanding the system dynamics and monitoring the state of the environmental health, detecting the possible pollution and its sources. The aims of this study, which is the first of its kind, are therefore i) to preliminarily investigate MP pollution in karst systems, from surface watercourses to cave waters, to provide a reference for further research, and ii) to discuss the abundance, morphological characteristic and types of MPs in the karst system.

2. Materials & Method

2.1. Study area

The Bossea karst system feeding area (Piedmont, NW Italy) (Fig. 1) is characterised by a belt of carbonate rocks laterally confined by poorly permeable rocks (quartzites and meta-volcanics) (Fig. 2). Bossea Cave (Frabosa Soprana (CN), Piedmont, Italy) represents the final part of the karst system developing in the Maudagna-Corsaglia watershed, between the Prato Nevoso basin and the Corsaglia River (Fig. 2). The land cover is mostly composed of broadleaf forests of larches, alternated with mountain meadows and pastures, although in a part of it, there are several ski slopes which attract many tourists in winter (Fig. 2A).

The recharge of this aquifer (Fig. 2B) is mainly linked to primary and/or secondary inputs: the primary inputs (or authigenic) are by rain or snow melt, directly recharging the aquifer, whereas the secondary inputs (or allogenic) of concern are the surface runoff waters continuously recharging the aquifer trough sinkholes in the sub-riverbed (Vigna, 2020). Rio Roccia bianca is the main secondary supply contributing to the Bossea karst system recharge (Fig. 2). A series of tests were previously carried out in the two main absorbent valleys (Rio Roccia bianca and Rio Bertino) (Vigna, 2020), using two different dyes (Tinopal and fluorescein), to examine their arrivals in Bossea Cave. This aquifer has a water circulation along karst conduits and fractures, with an extensive and well-developed saturated zone, and can therefore be described as a system with interconnected drainage, with a medium-high permeability (Banzato et al., 2011).

Bossea Cave is the first show cave of Italy, open to the public since 1874. The cavity has a single entrance at 836 m a.s.l. and an ascending structure (Fig. 3), with a 2,800-m development in the tectonic contact between the Middle Triassic Dolomie di San Pietro dei Monti formation (dolostone and carbonate rocks) and Permotriassic Metavolcanics (Antonellini et al., 2019). Different underground karst laboratories to study and monitor hydrogeology, subterranean meteorology, radon activity and subterranean biology are located in the cave (touristic and non-touristic areas), managed by Struttura Operativa Bossea C.A.I., Biologia Sotterranea Piemonte – Gruppo di Ricerca and the Department of Environment, Land and Infrastructure Engineering (DIATI) of the Politecnico di Torino, working together with ARPA Piemonte. Automatic detectors were installed in different parts of the cave to continuously (hourly) monitor water quantitative and qualitative data such as flow rate (Q), electrical conductivity (EC) and temperature (T). The flow rate is calculated through overflow vents or calibrated flow sections, depending on the water levels. Bossea Cave offers an ideal situation to study the groundwater circulation in a carbonate rock mass: a main water collector, called Mora River, and several water supplies with different flow rates, related to rock fractures, are present (Fig. 3B). Continuous monitoring data carried out in Bossea Cave by the DIATI - Politecnico di Torino team - revealed that the collector has a flow rate ranging from 50 to 1,200 L/s, developing for about 1.5 km in the cave and directly flowing in the Corsaglia River, with a set of springs (Banzato et al., 2011; Fiorucci et al., 2015; Fiorucci and Vigna, 2015; Peano et al., 2011; Vigna et al., 2017). The discontinuities are poorly or not karstified, characterised by low flow circulation ranging from 0.01 to 2.5 L/s (pools of water or drips), such as Polla delle anatre, one of the most important monitoring points in the Bossea Cave, tracked for over 40 years, with a highly constant flow rate (0.5 to 2.5 L/s) (Balestra et al., 2022a; Vigna et al., 2017). Moreover, secondary inputs continuously recharging the aquifer were previously monitored, proving their arrivals in Bossea Cave and highlighting the surface and underground environment connections. Finally, as the hydrodynamic response to rainfall events is characterised by a rather rapid increase in flow (Banzato et al., 2011), the movement of pollutants from the surface to the underground environments could be rapid, making this type of karst systems more subject to MP pollution.

2.2. Field sampling

For data collected in flowing water, it is essential to take into account the water flow rate to evaluate the correct MP percentage, which clearly depends on the water flow. Moreover, the season could affect the concentration of MPs in the water. Hence, sampling at different times could be performed to understand the impact of the season. However, these first samples taken punctually in the karst system are important to verify the presence of MPs in surface and subterranean waters; these data are scarce in karst areas and absent in caves. In MPs occur in each part of the system, subsequent analyses will be carried out to verify the impact of the seasons.

Sampling points were chosen to check pollution, starting from the surface water streams that recharge the karst system examined up to the springs, passing through the underground structures that accumulate in the water reserve and, therefore, can retain and/or transport pollutants.

One surface water sample was taken from Rio Roccia bianca, upstream the Bossea Cave (Fig. 2), four groundwater samples were collected from different areas of the Bossea Cave (Figs. 2–3, Table 1), and one surface water sample was taken from the Corsaglia River, where the water of the collector emerges (Fig. 2). In the Bossea Cave, two samples were collected nearer to the tourist path (Uovo and Sala frane) and two in different non-tourist areas (Sifone and Polla delle anatre) (Fig. 3, Table 1) as reference samples of unpolluted waters because these areas are not frequented by tourists or in close contact with human activities (Fig. 3, Table 1). In the tourist section, the collector water flows from the innermost areas towards the entrance of the cave, where the Sala frane sampling area is located. In this zone, the collector water flows quickly before leaving the cave. In certain areas of the cave, landslides and speleothems convey a small amount of this water along secondary paths, feeding dams of various sizes and slowing the flow, such as in the Uovo sampling area (Fig. 3, Table 1). Here, water flows between different gours, a particular type of speleothem (cave formation) in the form of a stone dam (Hill and Forti, 1997), adjacent to the tourist route, where an egg-shaped statue is placed (Fig. 3, Table 1). In Sifone, the water comes out from the rock, from below, in a siphoning section, and accumulates in a lake (Fig. 3, Table 1). It is possible to access the lake only by a rubber dinghy, in a speleological section of the cave. Polla delle anatre is a small pool of water entering in the inner collector, located in a non-touristic cave zone, visited only by researchers and speleologists (Fig. 3, Table 1). This water flows out of a fracture in the rock and is collected in a small tank with a weir for flow monitoring. Polla delle anatre flow has the typical characteristics of an interconnected drainage system, with a flow rate ranging from 0.5 to 2.5 L/s (Balestra et al., 2022a). Groundwater and surface water samples were collected in September 2021 under low-flow conditions. Being a preliminary investigation, water samples were collected into 1-L glass vessels and capped immediately to prevent atmospheric contamination. All sampling materials were pre-cleaned with ethanol and distilled water to avoid contamination. All samples were transported to the analytical laboratories of the Politecnico di Torino in ice-filled coolers and kept refrigerated at 6°C until analysis.

2.3. Laboratory analysis

Microplastic samples were processed according to the previously published method for cave sediments (Balestra and Bellopede, 2022b), adapted for the liquid matrix and improved to determine the particles using the infrared (IR) spectroscopy. All researchers used nitrile gloves and cotton coats in all steps, and all surfaces and materials used during sampling and laboratory analysis were cleaned with ethanol and distilled water to avoid MP contamination. Plastic equipment was replaced with glass or metal utensils.

Each water sample was filtered through an 0.8-µm pore size silver filter (GVS Life Sciences, Membrane Disk 47 mm) to allow a better analysis by IR spectroscopy. Filters were placed on glass petri dishes covered with aluminium foil and dried in an oven at 40°C for 2 h. As previous studies have shown the importance of organic matter removal (e.g. Balestra and Bellopede, 2022b), dried filters were subjected to this fundamental step through the application of 2 mL of 15% hydrogen peroxide solution, left to react for 30 min at room temperature and dried again for 2 h at 50°C.

Fluorescent whitening agents (FWAs) are often used in the production of plastic (Qiu et al., 2015), and MPs with FWAs can be easily detected under ultraviolet (UV) light (Ehlers et al., 2020; Klein and Fischer, 2019). Microplastics on filters were observed alongside with and without an UV flashlight (Alonefire SV10 365 nm UV flashlight 5W) under a Leitz ORTHOLUX II POL-MK microscope equipped with a DeltaPix Invenio 12EIII 12 Mpx Camera, with a 2.5x, 4x, 10x or higher magnification. As some particles are difficult to distinguish from natural ones, especially fibres, comparisons with images of natural, artificial and synthetic fibres under the microscope, taken in previous works (e.g. Houck, 2009; Khan et al., 2017; Zhang, 2014), were made. The UV flashlight was positioned on a pedestal with an inclination of 45°. Visual identification was performed to identify MPs in agreement with the strict selection criteria reported in previous works (Crawford and Quinn, 2016; Hidalgo-Ruz et al., 2012; Noren, 2007): fibres must have the same diameter along their entire length, particles must have unvarying colours, no cellular or organic structures must be present, and red, transparent or white particles must be examined under a high-magnification or fluorescence microscope. Particles smaller than 0.1 mm were cut off as suggested by the European Commission (2013)) for visual identification under a microscope. Particles that could not be identified as MPs were not take into consideration. Filters were observed by the same laboratory operator to ensure MP quantification and identification and reduce human errors. The MPs were categorised in relation to shape, size and colour, according to the standardised size and colour sorting system (SCS) proposed by Crawford and Quinn (2016)).

Usually, an average from 1% to 10% of the sample is analysed to determine the chemical composition of the MPs (International Organization for Standardization and European Committee for Standardization, 2020). Randomly, 10% of MPs of each filter were analysed using a micro-Fourier Transform Infrared Spectroscope (micro-FTIR) Shimadzu AIM-9000 microscope equipped with a Shimadzu IRTracer-100 spectrophotometer. Spectra were compared with the Shimadzu standard library.

3. Results and Discussion

Microplastics were found in all water samples, highlighting MP pollution in the Bossea karst system, from surface watercourses to underground waters. Although for data collected in flowing water, it is essential to take into consideration the water flow rate and the season to evaluate the correct MP abundance over time, this first investigation in the karst area is important because it highlights the presence of MPs in the surface and underground waters, even in areas not directly affected by human activities. Taking into account the continuous monitoring data carried out in the Bossea Cave by the DIATI, it is possible to associate the number of MPs to the Bossea collector (70 L/s) and Polla delle anatre (0.5 L/s) flow rate in September 2021.

3.1. Microplastic abundance

Microplastics were found in all water samples, including the two samples collected in the non-touristic areas of the Bossea Cave. Microplastic abundance, shape, dimension and fluorescence are shown in Table 2. The concentration of MPs in cave water varied from 12 to 54 items/L, with a mean of 28 items/L. The MP abundance in the surface water of Rio Roccia bianca was 23 items/L, whereas in the Corsaglia River, it was 29 items/L (Table 2). Because there is no standardised method for assessing MPs in water bodies, comparisons are challenging since measurement methods and treatments can affect the results. However, some assumptions on the amounts of MPs in karst environments can be made. Comparisons with other show cave water MP abundances are not possible at the moment as this study is the first one dealing with cave waters; however, a comparison with other groundwater and surface water samples from different areas of the world is possible (Table 3). To the authors´ knowledge, the only work on a karst area, monitoring springs and wells from two karst aquifers in Illinois, USA, documented the presence of a maximum MP concentration of 15.2 items/L (mean of 7.9 items/L) in springs and a maximum concentration of 4.4 items/L (mean of 2.8 items/L) in wells, under low-flow conditions (Panno et al., 2019). Selvam et al. (2021)) analysed 24 groundwater samples from wells and bore wells, collected in the post-monsoon season in coastal south India, pointing out a median concentration of 4.2 items/L, with a maximum concentration of 10.1 items/L. Other values are reported in Mintenig et al. (2019)) for NW Germany groundwater, with

5.6 items/L. Much higher values were found in an alluvial unconfined groundwater aquifer in Victoria, Australia, with an average concentration of 38 ± 8 items/L (Samandra et al., 2022). Very low values (1 items/L) were found in chalk and sandstone aquifers of the UK (Johnson et al., 2020). Surface water samples in the same studied aquifers were analysed by Selvam et al. (2021)), showing 7.8 items/L, with a maximum of 19.9 items/L., whereas Mintenig et al. (2019)) reported 2.9 items/L and Samandra et al. (2022)) 0.4 items/L. The microplastic abundances found in our study are high compared to those found in subterranean and surface waters of Northwest Germany (Mintenig et al., 2019), Illinois, USA (Panno et al., 2019) or South India (Selvam et al., 2021), whereas they are lower than those detected in groundwaters examined in Victoria, Australia (Samandra et al., 2022). However, it should be considered that the size of MPs taken into account, the sampling point, the flow rate, the season and the sources of MPs in the catchment may impact the concentrations of MPs in waters.

In non-tourist areas of the cave, the MP abundance in waters was similar to that in tourist areas, and a high number of MPs in the karst system water was found in the inner area of the cave, where the water comes out of a syphoning area. However, the Bossea Cave sediments collected in tourist areas contained more than twice as many MPs as those collected in non-tourist areas, suggesting that pollution is related to the passage of tourists. The microplastic presence in cave water, including water coming out of the rock in the non-tourist sections (Sifone and Polla delle anatre), suggests pollution linked to hydrogeologic connections from the surface to the underground aquifers. The high presence of fractures in the rocks of this karst area could favour pollutant transport in the underground environment, as suggested in Panno et al. (2019). It is reasonable to assume that the high degree of karstification in different zones, the presence of a well-organised outflow network and the plenty water flows play a fundamental role in the transport of MPs in this karst system. The contact between water and sediments could increase the MP concentrations in the Bossea karst system water. Microplastic pollution in the soil of this karst area could be related to the intense winter and summer activities in the zones above the karst system, and the area is rich in ski resorts (Prato Nevoso country). The presence of waste in these areas is not unusual, possible sources include plastic litter from anthropogenic activities and synthetic fibres from clothes. In these areas, surface waters that feed Rio Roccia bianca and Rio Bertino streams were collected, the main secondary supplies contributing to the Bossea karst system recharge (Vigna, 2020). A series of tests with dyes, previously carried out in the two main absorbent valleys, indicated that the waters coming from Rio Roccia bianca and Rio Bertino arrive in Bossea Cave in a relatively short time. During lean periods, the Rio Roccia Bianca waters arrive in the Bossea Cave collector after 4 days (speed of about 644 m/day), whereas in flood periods, they arrive after only 1 day (speed of about 2,460 m/day). Moreover, Rio Roccia bianca provides a much higher quantity of water to the Bossea systems than Rio Bertino water. Although currently, there are no studies studies on the atmospheric transport of pollutants in this area, soil pollution could be related to or aggravated by the atmospheric transport of particles depositing on the ground thanks to precipitation and wind as well as any other contamination between adjacent environments, linked to human activities from the near countries of Fontane and Prato Nevoso. In underground environments, tourists are carriers of alien particles such as lint, organic matter, dust and pollutants (Addesso et al., 2022; Balestra and Bellopede, 2022b; Jablonsky et al., 1993; Liu et al., 2021; Puławska et al., 2021), and therefore, waters in the tourist parts of the cave (Uovo and Sala frane) could be enriched with pollutants, such as MPs in cave sediments (Balestra and Bellopede, 2022b), which could be transported into different parts of the cave and outside, in the Corsaglia River. Our data do not show a definitive link to the suggested sources of pollution but offer information about the hydrogeologic connections, showing opportunities for future research on MP dynamics in subterranean waters in karst environments.

3.2. Microplastic shape and size

Microplastics were categorised according to the standardised size and colour sorting system (SCS) described in Crawford and Quinn (2016)) (Table 2). Fibres represented the majority of the MPs present in cave and surface waters (17.1% between 1 and 5 mm and 78% between 0.1 and 0.99 mm of length) (Figs. 4–5 and Table 2), followed by fragments (4.3% micro-fragments) (Fig. 5 and Table 2). Some microbeads (0.6%) were also observed, whereas no foam and films were found in this karst system (Fig. 5 and Table 2). As in previous research, fibres and fragments are the main shapes present in the groundwaters, albeit at different concentrations, such as 94% fragments (Samandra et al., 2022) or 100% fibres (Panno et al., 2019). In this work, fibre-shaped fragments dominated the samples. As more than 60% of the world textiles are produced from synthetic fibres (Henry and Klepp, 2018), synthetic clothes are the main source of MP pollution in this karst system. The fragment source could be linked to human activity on the surface or the presence of degraded waste in soil or could have been produced during the electric system works in the cave.

According to Crawford and Quinn (2016)), MPs are categorised into microplastics (1–5 mm) and mini-microplastics (1 mm–1 µm). Using visual identification under a microscope facilitated the analysis of MP particles from 5 to 0.1 mm. In this investigation, mini-MPs accounted for 82.9% of the total MPs found in the water karst system and for 84% of the MPs found in cave water only (Fig. 5 and Table 2).

The microplastic shape and size percentages found in Bossea Cave waters were similar to those described for cave sediments (Balestra and Bellopede, 2022a; Balestra and Bellopede, 2022b). However, the means of transport and the source could be different. Microplastic found in the Bossea Cave sediments could be mainly related to the passage of tourists along the cave path and the speleologists crossing the non-tourist passage to reach the internal laboratory (Balestra and Bellopede, 2022b). In contrast, the sources of MPs in the cave waters can be linked to cave tourism and pollution of the land above. The sources and transport of MPs in the cave should be studied in more detail to better understand MP pollution in karst areas.

As suggested in Panno et al. (2019)), the relative size of MP particles offers some insight into their movement. The MPs found in underground waters in this study were similar to those collected in the surface waters. In this karst area, water migrates from the surface to the underground environments via sinkholes and rapidly enters the fractures and crevices of the underlying aquifers, reaching the cave and finally discharging into the Corsaglia River. Through the soil pores and along irregular fractures and crevices, the larger MPs would be impeded, and only the smaller MP fractions would reach and migrate through the karst aquifers.

3.3. Microplastic fluorescence and colour

The highest MP abundance was fluorescent under UV light (84.8%) (Fig. 4, Table 2), with a percentage similar to that found for MPs in cave sediments (87.7%) (Balestra and Bellopede, 2022a; Balestra and Bellopede, 2022b). Exploiting fluorescent whitening agents (FWAs) in plastics, MPs can be easily found using an inexpensive UV flashlight (Fig. 4); however, there are also several limitations (Qiu et al., 2015): not all plastics contain FWAs (Fig. 4A-B, orange arrow), and different naturally fluorescent organic and inorganic matter compounds could be misunderstood as MPs. Organic fluorescent particles may account for more than half of the total fluorescent particles in the samples analysed in cave environment (Balestra and Bellopede, 2022b), and therefore, organic matter removal is a fundamental step in MP determination.

Figures 6 shows the different colours of the collected MPs. Of the fluorescent particles (Fig. 6A), 46% were transparent, followed by red (16.5%), blue (10.8%), beige (7.2%) and amber (4.3%) ones. The MPs with other colours accounted for less than 4% of the total fluorescent ones. Non-fluorescent MPs (Fig. 6B) were mainly black (68%) or blue (20%), followed by green, transparent and violet ones (4%). The major part of the fluorescent particles in water are transparent, as reported in Balestra and Bellopede (2022b)) for Bossea Cave sediments. The other main colours are similar, with the exception of red particles, which are more abundant in water. The non-fluorescent MP particles were mainly black and blue, confirming the data found for Bossea Cave sediments (Balestra and Bellopede, 2022b). However, the percentages were different, as well as the colour of the less abundant MPs. These facts could be related to the presence of particles with different densities, more easily transportable in water, with a different degree of biodeterioration or a diverse pollution source. Transparent MPs were also prevalent in groundwaters analysed in Selvam et al. (2021)), followed by white and blue ones, whereas in Panno et al. (2019)), 65% of the analysed MPs were blue and/or clear, whereas the other common colours were red (15%) and grey (13%).

The MP colour could provide an indication of the chemical pollutants; for example, yellow and black MPs could indicate high levels of pollutants (Frias et al., 2010; Karapanagioti et al., 2011). In the Bossea karst system, about 68% of non-fluorescent MPs were black, and future investigations should be carried out to monitor possible environmental contaminations. Moreover, the colour of MPs can be correlated with the consumption by organisms (Carpenter et al., 1972; Romeo et al., 2015; Shaw and Day, 1994), being confused with trophic resources. In natural subterranean systems, there is no light, and therefore, the colour of MPs does not play a particular role in hypogeal animal ingestion. However, the colour and fluorescence of MP particles may be relevant for less cave-adapted and epigeal organisms in watercourses and adjacent habitats.

3.4. Microplastic characterisation

Visual identification is one of the most inexpensive and commonly used methods for MP detection (e.g. Alomar et al., 2016; Cannas et al., 2017; Guerranti et al., 2017; Hidalgo-Ruz et al., 2012). However, it does not allow the determination of small particles (European Commission, 2013), is susceptible to operator experience and errors (Crawford and Quinn, 2016; Prata et al., 2019) and does not allow conclusions about polymer composition. In-depth analyses can be done with high-magnification microscopes, staining techniques and via spectroscopy. However, over- or underestimation of MPs can occur for different techniques (Hidalgo-Ruz et al., 2012; Song et al., 2015), and dyes have different limitations related to MP particle shape, typology, hydrophobicity and superficial alteration (Erni-Cassola et al., 2017; Prata et al., 2019; Shim et al., 2016; Tamminga et al., 2017). Reasonably, a set of several methodologies is the optimal choice, and therefore, in this work, spectroscopy was selected to confirm the results obtained via visual counting under the microscope.

The identification of MPs by spectroscopy is time-consuming, and generally, only an average from 1% to 10% of the sample is analysed (International Organization for Standardization and European Committee for Standardization, 2020). Moreover, the spectra of samples are difficult to match with the library ones, obtaining high percentages because of the contaminated surface of plastics found in natural environments (Song et al., 2015). In this study, 10% of MPs in each filter was analysed by micro-FTIR, taking random MP particles (Fig. 7). Polyethylene (51.4%) and polyvinyl alcohol (31.4%) were the main types of MPs found in the karst system waters, followed by polyester (8.6%), ethylene vinyl alcohol (2.9%), polyvinyl chloride

(2.9%) and acrylic adhesive (2.9%). Considering cave groundwaters only, polyethylene (56.5%) and polyvinyl alcohol (30.4%) were the main types of MPs. Polyethylene, polyester and polyvinyl chloride were also found in other analysed groundwaters (Mintenig et al., 2019; Panno et al., 2019; Samandra et al., 2022; Selvam et al., 2021). Other different MPs were identified in previous works, such as polyamide (nylon) (Mintenig et al., 2019; Samandra et al., 2022; Selvam et al., 2021), epoxy resin (Mintenig et al., 2019), polypropylene, polystyrene, polycarbonate, polyethylene terephthalate and polymethylmethacrylate (Samandra et al., 2022). The MP composition is useful to understand the likely sources of pollution. Many of the plastics found are used in the production of textiles, corroborating earlier assumptions on the origin of microplastics found in the waters of the karst system.

Anyway, natural, semi-synthetic, synthetic fibres and different types of MPs contain chemical additives such as dyes, whitening agents and other pollutants (Barker, 1975; Lee et al., 2020; Luo et al., 2019), which can be discharged into the environment and ingested by aquatic organisms (McNeish et al., 2018). Therefore, further studies on the different pollutants associated with MPs and textile fibres are necessary to quantify them and verify the possible environmental impacts.

4. Conclusions

This preliminary investigation documents the presence of microplastics in the examined karst system, from surface watercourses to cave waters. The concentration of MPs in the karst system water varied from 12 to 54 items/L. Based on the analysis of karst waters, microplastics less than 1 mm dominated the samples (82.9%), and fibre was the main shape present (95.1%). The detection of MP contamination is essential to define the issues related to pollution and establish strategies for karst areas and water conservation. This work suggests that karst morphology and associated aquifers could allow the movement of microplastics into underground water flow systems, providing useful references for further research. Future studies are needed to understand microplastic dynamics and transport in the karst system, to determine pollution sources and to understand the potential effects on organisms and ecosystems. As karst aquifers are open systems susceptible to contamination by surface pollutants, the superficial areas should also be monitored. Greater efforts should be made to protect karst areas and underground resources, implementing new strategies to monitor and valuate MP pollution and providing education to all stakeholders to find adequate solutions, following the environmental sustainability principles, especially for the management of water resources.

Acknowledgements

The authors are grateful to the reviewer for the critical review of the manuscript, the Bossea Cave managers, Claudio De Regibus (Politecnico di Torino) for helping with laboratory equipment, Davide Janner and Matteo Giardino for spectroscopy advices and Micro-FTIR use, Stefano Vanin (University of Genova) for providing us with forensic science materials, Sinem Akyildiz (Marmara University) for providing the textile engineering books, Fabrizio Bianco (Politecnico di Torino) for assisting in sampling and Maria Sighincelli (ENEA) and Adriano Fiorucci (Politecnico di Torino) for useful suggestions.

Funding: This work was realised within the research project "SHOWCAVE: a multidisciplinary research project to study, classify and mitigate the environmental impact in tourist caves", funded by the Italian Ministry of Education, University and Research [PRIN: Progetti di ricerca di rilevante interesse nazionale 2017 - Prot. 2017HTXT2R; PI: Prof. Marco Isaia, University of Torino].

CRediT authorship contribution statement

Valentina Balestra: Conceptualisation, methodology, validation, formal analysis, investigation, resources, data curation, writing – original draft, writing – review and editing, visualisation. Bartolomeo Vigna: Investigation, writing – review and editing, visualisation. Sean de Costanzo: Investigation. Rossana Bellopede: Conceptualisation, methodology, validation, resources, writing – review and editing, visualisation, project administration, funding acquisition.

References

Addesso, R., Pingaro, S., Bisceglia, B. and Baldantoni, D., 2022. Sustainable Tourism and Conservation of Underground Ecosystems through Airflow and Particle Distribution Modeling. Sustainability, 14(13): 7979.

Allen, S. et al., 2019. Atmospheric transport and deposition of microplastics in a remote mountain catchment. Nature Geoscience, 12(5): 339-344.

Alomar, C., Estarellas, F. and Deudero, S., 2016. Microplastics in the Mediterranean Sea: deposition in coastal shallow sediments, spatial variation and preferential grain size. Marine environmental research, 115: 1-10.

Ambrosini, R. et al., 2019. First evidence of microplastic contamination in the supraglacial debris of an alpine glacier. Environmental pollution, 253: 297-301.

Antonellini, M., Nannoni, A., Vigna, B. and De Waele, J., 2019. Structural control on karst water circulation and speleogenesis in a lithological contact zone: The Bossea cave system (Western Alps, Italy). Geomorphology, 345: 106832.

Assas, M. et al., 2020. Bioaccumulation and reproductive effects of fluorescent microplastics in medaka fish. Marine Pollution Bulletin, 158: 111446.

Balestra, V. and Bellopede, R., 2022a. Microplastic pollution in show cave sediments. In: S.G. Italiana (Editor), SGI-SIMP Congress "Geosciences for a sustainable future" Torino, pp. 548.

Balestra, V. and Bellopede, R., 2022b. Microplastic pollution in show cave sediments: First evidence and detection technique. Environmental Pollution, 292: 118261.

Balestra, V. et al., 2021. Study of the environmental impact in show caves: a multidisciplinary research. Geoingegneria Ambientale e Mineraria, 163-164: 24-35.

Balestra, V., Fiorucci, A. and Vigna, B., 2022a. Study of the Trends of Chemical–Physical Parameters in Different Karst Aquifers: Some Examples from Italian Alps. Water, 14(3): 441.

Balestra, V., Lana, E. and Vanin, S., 2022b. Observations on the habitat and feeding behaviour of the hypogean genus Eukoenenia (Palpigradi, Eukoeneniidae) in the Western Italian Alps. Subterranean Biology, 42: 23-41.

Banzato, C., De Waele, J., Fiorucci, A. and Vigna, B., 2011. Study of springs and karst aquifers by monitoring and geochemical analysis, Proceedings of the 9th H2 Karst Conference on Limestone Hydrogeology, Besançon, France, pp. 1-3.

Barker, R.H., 1975. Additives in fibers and fabrics. Environmental Health Perspectives, 11: 41-45.

Barzaghi, B., De Giorgi, D., Pennati, R. and Manenti, R., 2021. Planarians, a Neglected Component of Biodiversity in Groundwaters. Diversity, 13(5): 178.

Boyle, K. and Örmeci, B., 2020. Microplastics and nanoplastics in the freshwater and terrestrial environment: A review. Water, 12(9): 2633.

Braun, L.N., 1984. Simulation of snowmelt-runoff in lowland and lower alpine regions of Switzerland, ETH Zurich.

Cabrera, M. et al., 2020. A new method for microplastic sampling and isolation in mountain glaciers: A case study of one antisana glacier, Ecuadorian Andes. Case Studies in Chemical and Environmental Engineering, 2: 100051.

Cannas, S., Fastelli, P., Guerranti, C. and Renzi, M., 2017. Plastic litter in sediments from the coasts of south Tuscany (Tyrrhenian Sea). Marine pollution bulletin, 119(1): 372-375.

Carpenter, E.J., Anderson, S.J., Harvey, G.R., Miklas, H.P. and Peck, B.B., 1972. Polystyrene spherules in coastal waters. Science, 178(4062): 749-750.

Chauve, P., Mania, J. and Moindrot, D., 1990. Modalités de la fonte de neige en moyenne montagne et alimentation du karst sous-jacent. Hydrology in Mountainous Regions. I Hydrological Measurements; The Water Cycle(193).

Chia, R.W., Lee, J.-Y., Kim, H. and Jang, J., 2021. Microplastic pollution in soil and groundwater: a review. Environmental Chemistry Letters: 1-14.

Christman, A., 2019. Cave Dwelling Dust Bunnies: Lint Accumulation and Microplastics in Lewis and Clark Caverns State Park. PhD Thesis Thesis, Carroll College, Helena, Montana.

Cigna, A.A. and Forti, P., 2013. Caves: the most important geotouristic feature in the world. Tourism and Karst areas, 6(1): 9-26.

Cincinelli, A. et al., 2017. Microplastic in the surface waters of the Ross Sea (Antarctica): occurrence, distribution and characterization by FTIR. Chemosphere, 175: 391-400.

Corami, F., Rosso, B., Bravo, B., Gambaro, A. and Barbante, C., 2020. A novel method for purification, quantitative analysis and characterization of microplastic fibers using Micro-FTIR. Chemosphere, 238: 124564.

Crawford, C.B. and Quinn, B., 2016. Microplastic pollutants. Elsevier, Amsterdam.

Devereux, R., Hartl, M.G., Bell, M. and Capper, A., 2021. The abundance of microplastics in cnidaria and ctenophora in the North Sea. Marine Pollution Bulletin, 173: 112992.

Ehlers, S.M., Maxein, J. and Koop, J.H., 2020. Low-cost microplastic visualization in feeding experiments using an ultraviolet light-emitting flashlight. Ecological Research, 35(1): 265-273.

Elia, E. and Calleris, V., 1988. Grotta di Bossea, Mondo ipogeo, Cuneo, 5-10.

Erni-Cassola, G., Gibson, M.I., Thompson, R.C. and Christie, J.A., 2017. Lost, but found with Nile red; a novel method to detect and quantify small microplastics (20 µm–1 mm) in environmental samples 2.

European Commission, 2013. Guidance on monitoring of marine litter in European seas. A guidance document within the common implementation strategy for the Marine Strategy Framework Directive. Ispra: European Commission, Joint Research Centre, MSFD Technical Subgroup on Marine Litter, pp. 126.

Fahrenfeld, N., Arbuckle-Keil, G., Beni, N.N. and Bartelt-Hunt, S.L., 2019. Source tracking microplastics in the freshwater environment. TrAC Trends in Analytical Chemistry, 112: 248-254.

Fiorucci, A., Moitre, B. and Vigna, B., 2015. Hydrogeochemical study of Bossea karst system, Proceedings of the international symposium in environmental safety and construction in karst areas., Perm, Russia, pp. 290-294.

Fiorucci, A. and Vigna, B., 2015. Hydrogeochemical study of some springs in the ligurian and marittime alps (Piedmont, Italy), Proceedings of the second Russian scientific Conference Water-Rock interaction: Geological evolution, Vladivostok, Russky Island, FEFU Campus, Russia, pp. 229-233.

Ford, D. and Williams, P.D., 2013. Karst hydrogeology and geomorphology. John Wiley & Sons.

Ford, D.C. and Williams, P.W., 1989. Karst geomorphology and hydrology, 601. Unwin Hyman London.

Frei, S. et al., 2019. Occurence of microplastics in the hyporheic zone of rivers. Scientific reports, 9(1): 1-11.

Frias, J., Sobral, P. and Ferreira, A.M., 2010. Organic pollutants in microplastics from two beaches of the Portuguese coast. Marine pollution bulletin, 60(11): 1988-1992.

Guerranti, C. et al., 2017. Plastic litter in aquatic environments of Maremma Regional Park (Tyrrhenian Sea, Italy): Contribution by the Ombrone river and levels in marine sediments. Marine pollution bulletin, 117(1-2): 366-370.

Henry, B. and Klepp, I.G., 2018. Microplastic pollution from textiles: A literature review. Project Report no.1–2018, Oslo.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C. and Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environmental science & technology, 46(6): 3060-3075.

Hill, C. and Forti, P., 1997. Cave Minerals of the World (2nd Ed.). National Speleological Society, Huntsville, Alabama, U.S.A.

Hottelet, C., Braun, L., Leibundgut, C. and Rieg, A., 1993. Simulation of snowpack and discharge in an alpine karst basin. IAHS Publications-Publications of the International Association of Hydrological Sciences, 218: 249-260.

Houck, M.M., 2009. Identification of textile fibers.

International Organization for Standardization and European Committee for Standardization, 2020. Plastics - Environmental aspects - State of knowledge and methodologies (CEN ISO/TR 21960:2020).

Jablonsky, P., Kraemer, S. and Yett, B., 1993. Lint in caves, National Cave Management Symposium, Carlsbad, NM, pp. 73-81.

Jahan, S. et al., 2019. Interrelationship of microplastic pollution in sediments and oysters in a seaport environment of the eastern coast of Australia. Science of the Total Environment, 695: 133924.

Johnson, A.C. et al., 2020. Identification and quantification of microplastics in potable water and their sources within water treatment works in England and Wales. Environmental Science & Technology, 54(19): 12326-12334.

Karapanagioti, H., Endo, S., Ogata, Y. and Takada, H., 2011. Diffuse pollution by persistent organic pollutants as measured in plastic pellets sampled from various beaches in Greece. Marine Pollution Bulletin, 62(2): 312-317.

Khan, A., Abir, N., Rakib, M.A.N., Bhuiyan, E.S. and Howlader, M.R., 2017. A review paper on Textile Fiber Identification. IOSR Journal of Polymer and Textile Engineering (IOSR-JPTE), 4: 14-20.

Khant, N.A. and Kim, H., 2022. Review of Current Issues and Management Strategies of Microplastics in Groundwater Environments. Water, 14(7): 1020.

Klein, M. and Fischer, E.K., 2019. Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany. Science of the Total Environment, 685: 96-103.

Lee, Y.K., Murphy, K.R. and Hur, J., 2020. Fluorescence signatures of dissolved organic matter leached from microplastics: Polymers and additives. Environmental Science & Technology, 54(19): 11905-11914.

Li, J., Zhang, K. and Zhang, H., 2018. Adsorption of antibiotics on microplastics. Environmental Pollution, 237: 460-467.

Li, X. et al., 2019. Enhancement in adsorption potential of microplastics in sewage sludge for metal pollutants after the wastewater treatment process. Water Research, 157: 228-237.

Liu, K. et al., 2019. Source and potential risk assessment of suspended atmospheric microplastics in Shanghai. Science of the total environment, 675: 462-471.

Liu, Z. et al., 2021. Influence of the Visitor Walking on Airflow and the Bioaerosol Particles in Typical Open Tomb Chambers: An Experimental and Case Study. Buildings, 11(11): 538.

Luo, H. et al., 2019. Leaching behavior of fluorescent additives from microplastics and the toxicity of leachate to Chlorella vulgaris. Science of the Total Environment, 678: 1-9.

Lwanga, E.H. et al., 2017. Incorporation of microplastics from litter into burrows of Lumbricus terrestris. Environmental Pollution, 220: 523-531.

Manenti, R. et al., 2018. The stenoendemic cave-dwelling planarians (Platyhelminthes, Tricladida) of the Italian Alps and Apennines: conservation issues. Journal for Nature Conservation, 45: 90-97.

McGechan, M., 2002. SW—soil and water: transport of particulate and colloid-sorbed contaminants through soil, part 2: trapping processes and soil pore geometry. Biosystems Engineering, 83(4): 387-395.

McNeish, R.E. et al., 2018. Microplastic in riverine fish is connected to species traits. Scientific reports, 8(1): 1-12.

Mintenig, S., Löder, M., Primpke, S. and Gerdts, G., 2019. Low numbers of microplastics detected in drinking water from ground water sources. Science of the total environment, 648: 631-635.

Moindrot, P., Chauve, P. and Mania, J., 1988. Influence de l'enneigement sur l'hydrologie du bassin expérimental des Fourgs (Franche-Comté, France), Quatrième colloque d'hydrologie en pays calcaire et en milieu fissuré, Besançon, pp. 121-129.

Noren, F., 2007. Small plastic particles in Coastal Swedish waters, N-Research Report, commissioned by KIMO, Sweden.

Panno, S.V. et al., 2019. Microplastic contamination in karst groundwater systems. Groundwater, 57(2): 189-196.

Peano, G., Vigna, B., Villavecchia, E. and Agnesod, G., 2011. Radon exchange dynamics in a karst system investigated by radon continuous measurements in water: first results. Radiation Protection Dosimetry, 145(2-3): 173-177.

Prata, J.C. et al., 2019. A new approach for routine quantification of microplastics using Nile Red and automated software (MP-VAT). Science of the total environment, 690: 1277-1283.

Puławska, A., Manecki, M. and Flasza, M., 2021. Mineralogical and Chemical Tracing of Dust Variation in an Underground Historic Salt Mine. Minerals, 11(7): 686.

Qiu, Q. et al., 2015. Occurrence of microplastics in the coastal marine environment: First observation on sediment of China. Marine Pollution Bulletin, 98(1-2): 274-280.

Re, V., 2019. Shedding light on the invisible: addressing the potential for groundwater contamination by plastic microfibers. Hydrogeology Journal, 27(7): 2719-2727.

Rochman, C.M., Hoh, E., Kurobe, T. and Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific reports, 3(1): 1-7.

Romeo, T. et al., 2015. First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. Marine pollution bulletin, 95(1): 358-361.

Samandra, S. et al., 2022. Microplastic contamination of an unconfined groundwater aquifer in Victoria, Australia. Science of The Total Environment, 802: 149727.

Selvam, S., Jesuraja, K., Venkatramanan, S., Roy, P.D. and Kumari, V.J., 2021. Hazardous microplastic characteristics and its role as a vector of heavy metal in groundwater and surface water of coastal south India. Journal of Hazardous Materials, 402: 123786.

Sharma, S. and Chatterjee, S., 2017. Microplastic pollution, a threat to marine ecosystem and human health: a short review. Environmental Science and Pollution Research, 24(27): 21530-21547.

Shaw, D.G. and Day, R.H., 1994. Colour-and form-dependent loss of plastic micro-debris from the North Pacific Ocean. Marine Pollution Bulletin, 28(1): 39-43.

Shim, W.J., Song, Y.K., Hong, S.H. and Jang, M., 2016. Identification and quantification of microplastics using Nile Red staining. Marine pollution bulletin, 113(1-2): 469-476.

Sket, B., 1999. The nature of biodiversity in hypogean waters and how it is endangered. Biodiversity & Conservation, 8(10): 1319-1338.

Song, Y.K. et al., 2015. A comparison of microscopic and spectroscopic identification methods for analysis of microplastics in environmental samples. Marine pollution bulletin, 93(1-2): 202-209.

Tamminga, M., Hengstmann, E. and Fischer, E., 2017. Nile Red Staining as a Subsidiary Method for Microplastic Quantification: A Comparison of Three Solvents and Factors Influencing Application Reliability. Journal of Earth Sciences & Environmental Studies, 2(2): 165-172.

Van Cauwenberghe, L., Vanreusel, A., Mees, J. and Janssen, C.R., 2013. Microplastic pollution in deep-sea sediments. Environmental pollution, 182: 495-499.

Vardar, N. and Vidal Rodriguez, R., 2021. Assessment of Microplastic Debris in Coastal and Cave Environments, 4to Congreso de investigadores de la Universidad Interamericana de Puerto Rico.

Viaroli, S., Lancia, M. and Re, V., 2022. Microplastics contamination of groundwater: Current evidence and future perspectives. A review. Science of The Total Environment: 153851.

Vigna, B., 2007. Schematizzazione e funzionamento degli acquiferi in rocce carbonatiche. L'acqua nelle aree carsiche in Italia. Memorie dell’Istituto Italiano di Speleologia, 19(Serie II): 21-26.

Vigna, B., 2020. Assetto geologico ed idrogeologico del Sistema carsico di Bossea (SW Piemonte, Italy), Atti del Convegno Nazionale "L'uomo domanda, la grotta risponde". Cinquantesimo anniversario del Laboratorio Carsologico Sotterraneo di Bossea, Frabosa Soprana (CN), Italy, pp. 283-300.

Vigna, B. and Banzato, C., 2015. The hydrogeology of high-mountain carbonate areas: an example of some Alpine systems in southern Piedmont (Italy). Environmental Earth Sciences, 74(1): 267-280.

Vigna, B., Fiorucci, A., Nannoni, A. and De Waele, J., 2017. Vadose Zone Hydrogeology In The Bossea Cave System (Southern Piedmont, Northern Italy), Proceedings of the 17th International Congress of Speleology, Sidney, pp. 222-225.

Wanner, P., 2021. Plastic in agricultural soils–a global risk for groundwater systems and drinking water supplies?–a review. Chemosphere, 264: 128453.

White, W.B., 1969. Conceptual models for carbonate aquifers. Groundwater, 7(3): 15-21.

White, W.B., 1988. Geomorphology and hydrology of karst terrains. Oxford University Press, New York.

Wong, J.K.H., Lee, K.K., Tang, K.H.D. and Yap, P.-S., 2020. Microplastics in the freshwater and terrestrial environments: Prevalence, fates, impacts and sustainable solutions. Science of the total environment, 719: 137512.

Wright, S.L., Thompson, R.C. and Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environmental pollution, 178: 483-492.

Zhang, X., 2014. Fundamentals of fiber science. DEStech Publications, Inc.

Zhang, Y. et al., 2021. Microplastics in glaciers of the Tibetan Plateau: Evidence for the long-range transport of microplastics. Science of The Total Environment, 758: 143634.

Zhou, Y. et al., 2021. Microplastic contamination is ubiquitous in riparian soils and strongly related to elevation, precipitation and population density. Journal of Hazardous Materials, 411: 125178.

Zhou, Y., Liu, X. and Wang, J., 2019. Characterization of microplastics and the association of heavy metals with microplastics in suburban soil of central China. Science of the Total Environment, 694: 133798.

Figures

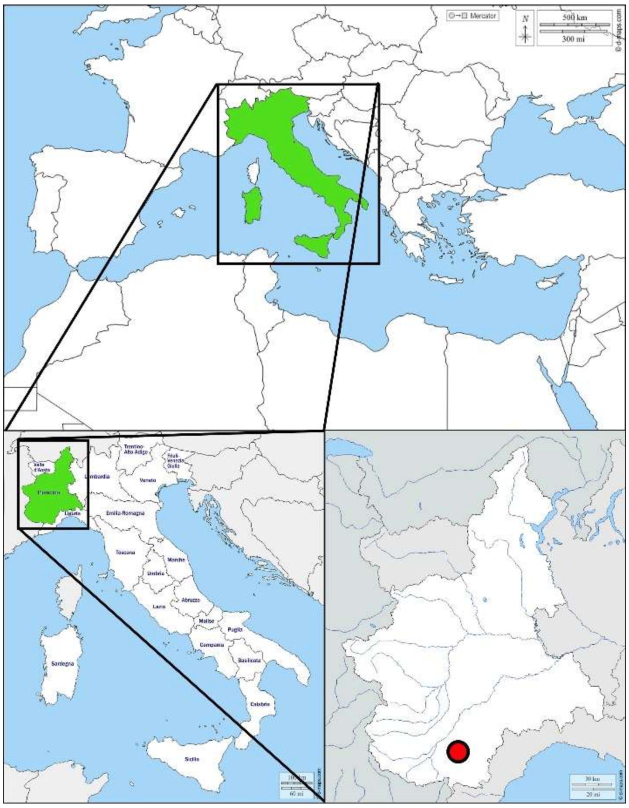

Fig. 1. Location of the sampling area. Bossea Cave is located in SW Piedmont (Italy). (Maps used for the plate and modified, retrieved from: https://d-maps.com/carte.php?num_car=3126&lang=en, https://d-maps.com/carte.php?num_car=4828&lang=it and https://d-maps.com/carte.php?num_car=8256&lang=en).

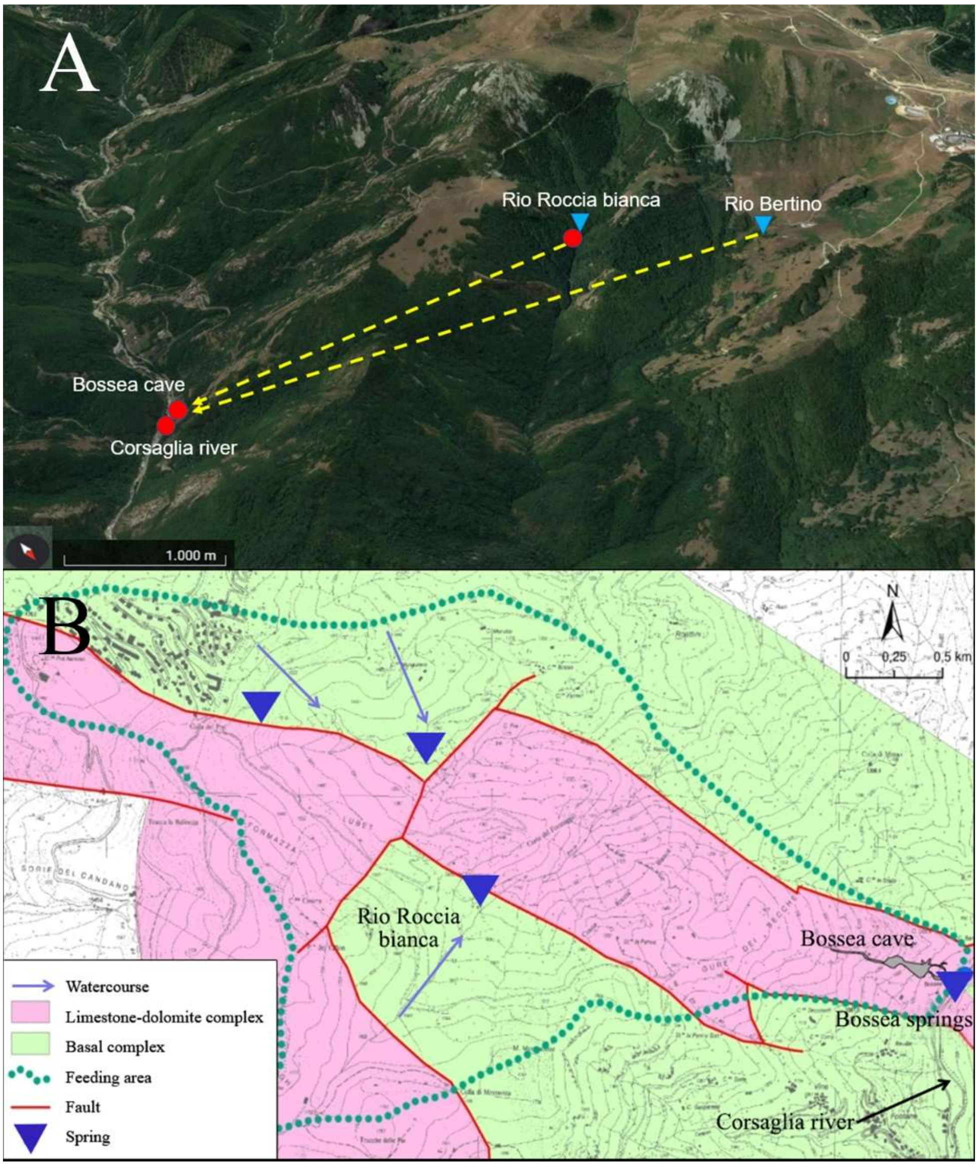

Fig. 2. Bossea karst system. A: Turquois triangles for the main surface water supply (Rio Roccia bianca and Rio Bertino), red circle for sampling areas, yellow arrow for the results of the staining tests carried out previously: the waters of the secondary supply contributing the Bossea karst system recharge (Rio Roccia bianca e Rio Bertino) trough sinkholes in the sub-riverbed, ending up in the collector of the Bossea Cave. Map from https://earth.google.com [accessed: 07/06/2022], modified; B: Bossea karst system feeding area. Map from Vigna (2020)), modified.

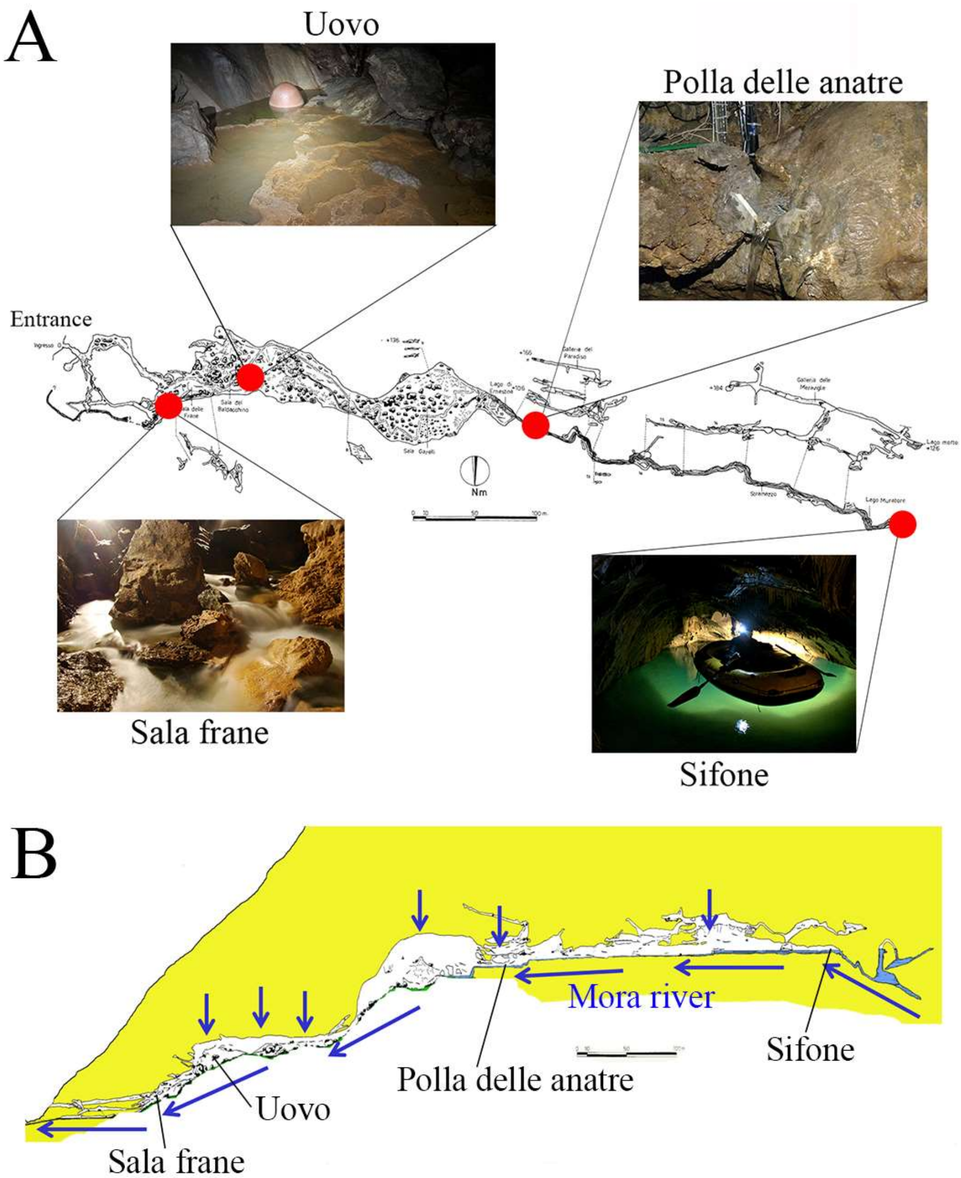

Fig. 1. Bossea karst system. A: Bossea Cave sampling areas. Red circles for water sampling. Survey (plan) by Elia and Calleris (1988)), modified. Photos by Valentina Balestra and Bartolomeo Vigna; B: Bossea Cave displacement of water with sampling areas. Blue arrows for primary and secondary water intakes. Survey (section) by Elia and Calleris (1988), modified).

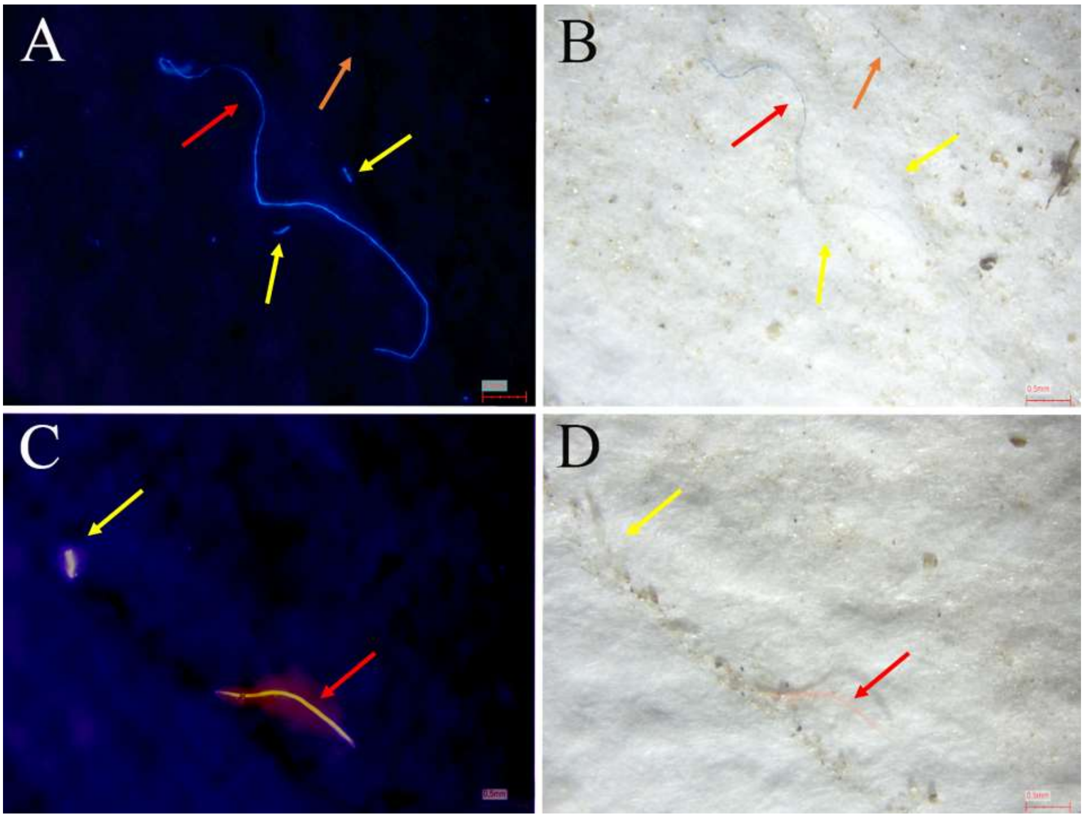

Fig. 2. Microplastic particles on filters observed under a microscope, with and without a UV flashlight. Yellow arrows for transparent microplastics, fluorescent under UV light, orange arrows for coloured microplastic, non-fluorescent under UV light, and red arrows for coloured microplastics, fluorescent under UV light. A–B: microplastic fibres; C–D: microplastic fibre and fragment.

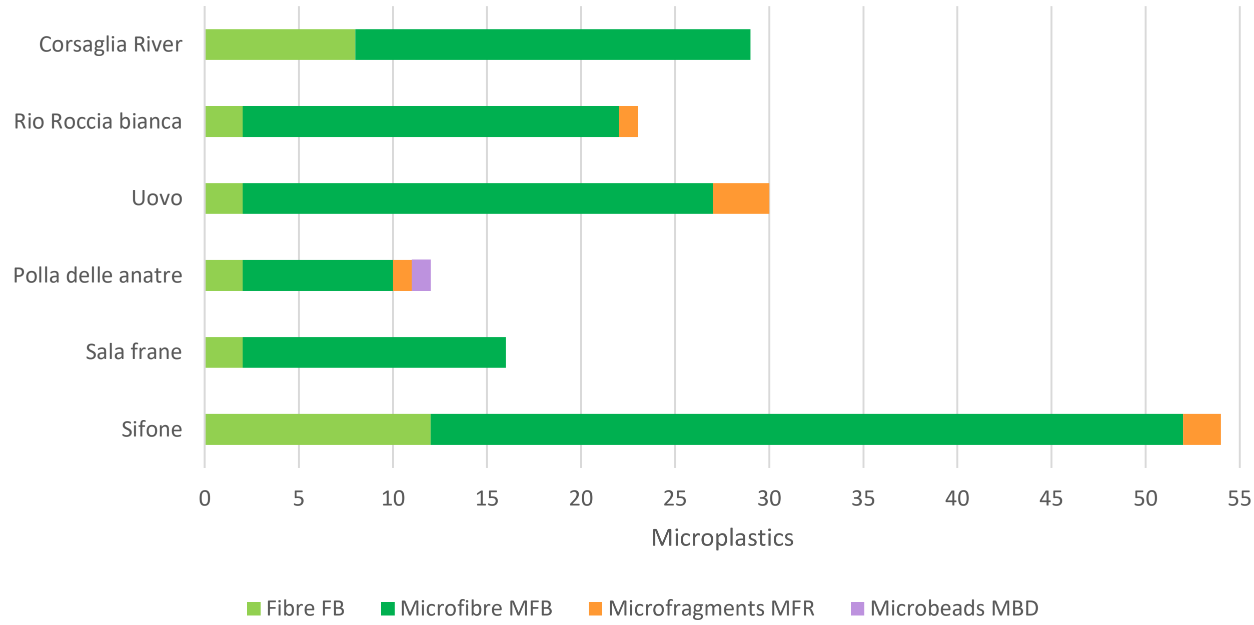

Fig. 3. Microplastic shape and size for the examined karst system waters.

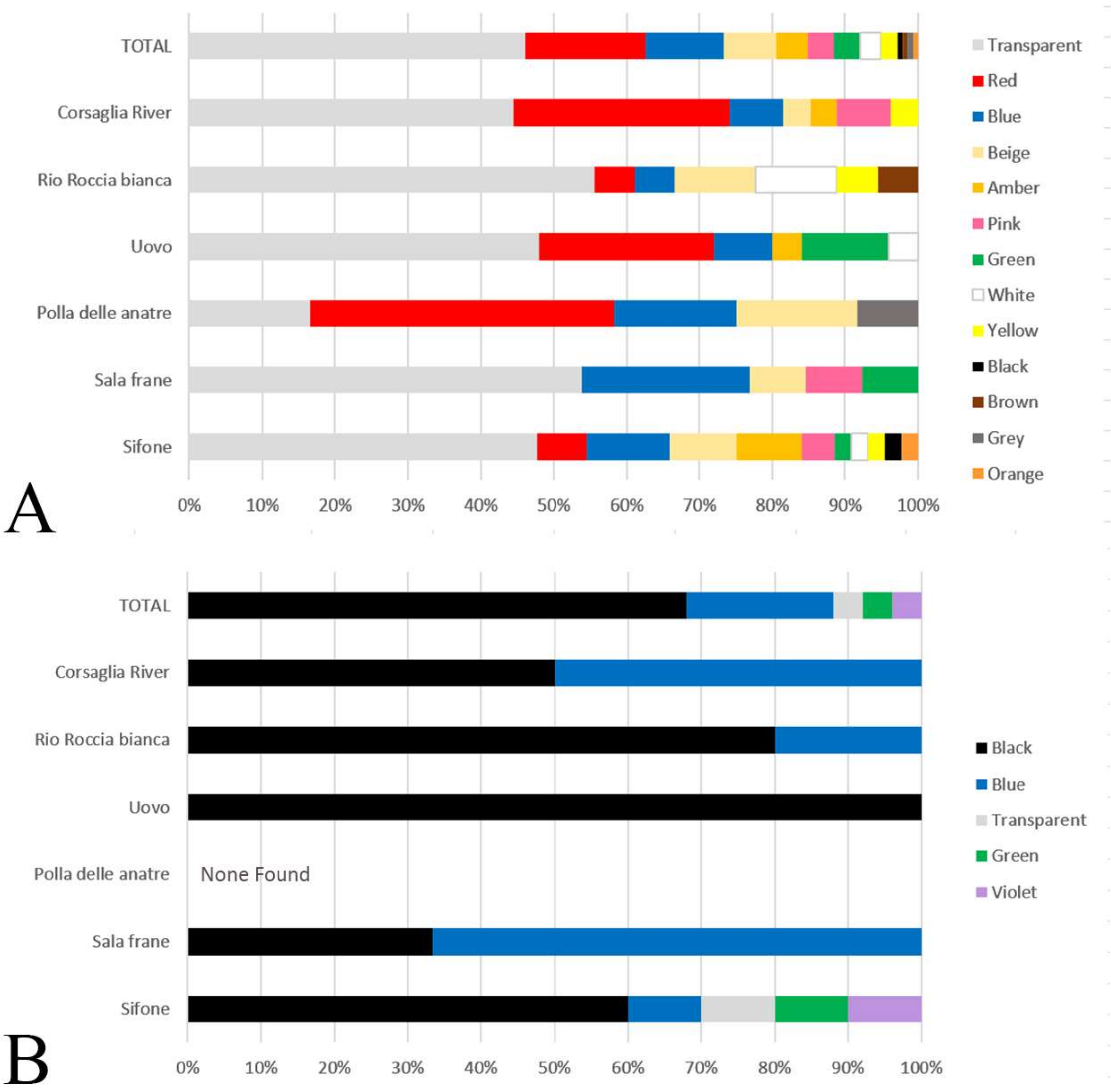

Fig. 6. Percentages of colours of microplastic particles on filters from water sampled in different Bossea karst system areas. Uovo, Polla delle anatre, Sala frane and Sifone were sampled in Bossea Cave. A: Percentages of colours of fluorescent microplastics. B: Percentages of colours of non-fluorescent microplastics.

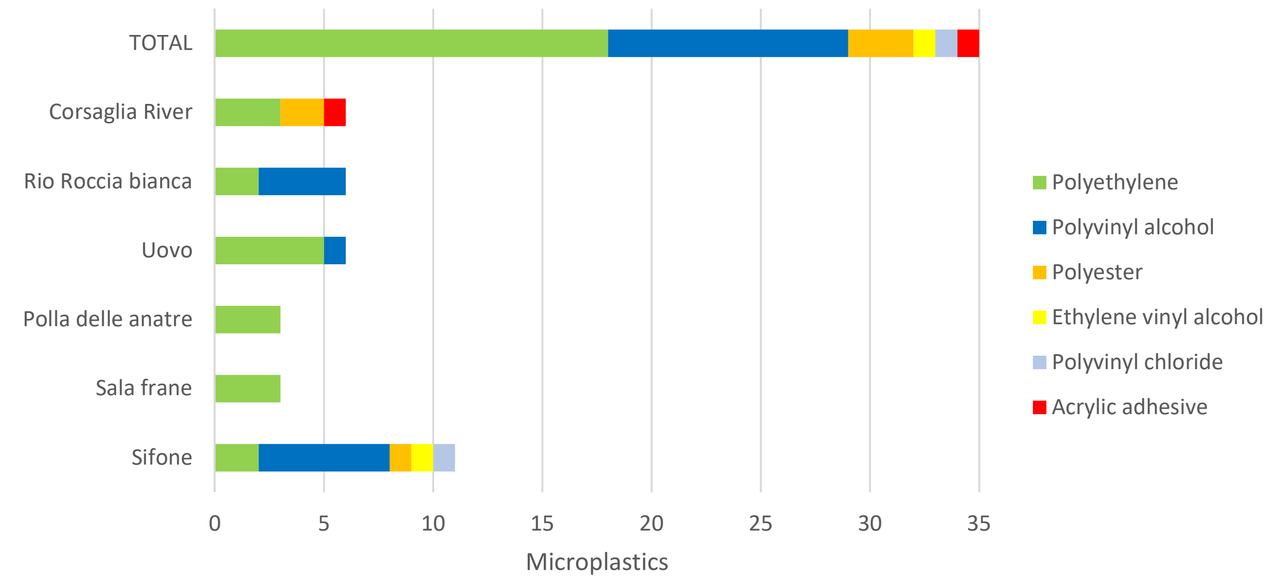

Fig. 7. Identification of 10% of the MP particles of each filter by micro-FTIR spectroscopy.

Tables

Table 1. Bossea Cave sampling area characteristics.

Sampling area	Distance from the entrance	Sampling site type	Water characteristics	Flow rate	Tourist/non-tourist area
Sifone	100 m	Spring	The water comes out from the rock, from below, in a siphoning section, and accumulates in a lake	-	Non-tourist area
Polla delle anatre	150 m	Small pool of water entering in the inner collector	The water flows out of a fracture in the rock and is collected in a small tank with a weir for flow monitoring. The flow has the typical characteristics of an interconnected drainage system	0.5 to 2.5 L/s	Non-tourist area
Uovo	400 m	A small amount of the collector's water conveyed by speleothems and landslides along secondary paths	The water flows slowly between different gours	-	Tourist area
Sala frane	700 m	Collector	The water flows quickly	50 to 1,200 L/s	Tourist area

Table 2. Abundance [items/L], shape, size and fluorescence of MPs in Bossea system waters. Shape and size were defined using the SCS Method (Crawford and Quinn, 2016).

Filter	MP	Fluorescent MPs	Non fluorescent MPs	Fibre FB	Microfibre MFB	Fragments FR	Microfragments MFR	Film FI	Microfilm MFI	Pellet PT	Microbeads MBD	Foam FM	Microfoam MFM
Sifone	54	44	10	12	40	0	2	0	0	0	0	0	0
Polla delle anatre	12	12	0	2	8	0	1	0	0	0	1	0	0
Uovo	30	25	5	2	25	0	3	0	0	0	0	0	0
Sala frane	16	13	3	2	14	0	0	0	0	0	0	0	0
Cave total	112	82	30	18	87	0	6	0	0	0	1	0	0

%	100	73.2	26.8	16.1	77.7	0.0	5.4	0.0	0.0	0.0	0.9	0.0	0.0
Rio Roccia bianca	23	18	5	2	20	0	1	0	0	0	0	0	0
Corsaglia river	29	27	2	8	21	0	0	0	0	0	0	0	0
Karst system total	164	127	37	28	128	0	7	0	0	0	1	0	0
%	100	84.8	15.2	17.1	78.0	0.0	4.3	0.0	0.0	0.0	0.6	0.0	0.0

Table 3. MP pollution comparison of groundwater and surface water samples from different areas of the world.

Reference	Country	Aquifer type	Groundwater sampling characteristics	Groundwater MP concentration	Surface water MP concentration
Mintenig et al. (2019))	Germany	-		5.6 items/L	2.9 items/L.
Panno et al. (2019))	USA	Karst aquifer	Spring and wells Low-flow conditions	Springs: Max 15.2 items/L Mean of 7.9 items/L Wells: Max 4.4 items/ Mean of 2.8 items/L	-
Johnson et al. (2020))	UK	Chalk and sandstone aquifer	-	1 items/L	-
Selvam et al. (2021))	India	Costal aquifer	Wells and bore wells Post-monsoon season	Max 10.1 items/L. Median 4.2 items/L	Max 19.9 items/L 7.8 items/L
Samandra et al. (2022))	Australia	Alluvial unconfined aquifer		Average 38±8 items/L Min 16 items/L Max 97 items/L	0.4 items/L