Microplastic pollution calls for urgent investigations in stygobiont habitats: A case study from Classical karst

Original
Microplastic pollution calls for urgent investigations in stygobiont habitats: A case study from Classical karst / Balestra, Valentina; Galbiati, Matteo; Lapadula, Stefano; Zampieri, Veronica; Cassarino, Filippomaria; Gajdošová, Magdalena; Barzaghi, Benedetta; Manenti, Raoul; Ficetola, Gentile Francesco; Bellopede, Rossana. - In: JOURNAL OF ENVIRONMENTAL MANAGEMENT. - ISSN 0301-4797. - ELETTRONICO. - 356:(2024), pp. 1-13. [10.1016/j.jenvman.2024.120672]

Availability:
This version is available at: 11583/2987260 since: 2024-03-23T21:28:32Z

Publisher:
Elsevier

Published
DOI:10.1016/j.jenvman.2024.120672

Journal of Environmental Management 356 (2024) 120672

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Microplastic pollution calls for urgent investigations in stygobiont habitats: A case study from Classical karst

Valentina Balestra [a,b,*], Matteo Galbiati [c], Stefano Lapadula [c], Veronica Zampieri [c], Filippomaria Cassarino [c], Magdalena Gajdošová [d], Benedetta Barzaghi [c], Raoul Manenti [c], Gentile Francesco Ficetola [c], Rossana Bellopede [a]

[a] *Department of Environment, Land and Infrastructure Engineering, Politecnico di Torino, Italy*
[b] *Biologia Sotterranea Piemonte - Gruppo di Ricerca, Italy*
[c] *Department of Environmental Science and Policy, Università degli Studi di Milano, Italy*
[d] *Department of Ecology, Faculty of Science, Charles University, Prague, Czech Republic*

ARTICLE INFO

Handling editor: Jason Michael Evans

Keywords:
Microplastic pollution
Aquatic environments
Troglobionts
Proteus anguinus
Caves
Springs

ABSTRACT

Microplastic pollution in karst systems is still poorly studied, despite the presence of protected species and habitats, and important water reserves. Vulnerable key species hosted in these habitats could consume or assimilate microplastics, which can irreversibly damage management efforts, and thus ecosystems functionality. This can be particularly true for subterranean water habitats where microplastic pollution effects on wildlife management programs are not considered. The aim of this study is to provide a case study from the Classical Karst Region, which hosts peculiar habitats and key species protected at European level, such as the olm *Proteus anguinus*. As this area has been deeply exploited and modified over time, and is adjacent to highways, roads and railways, which could contribute to pollution within the karst system, threatening the ecosystems, it provides a perfect model system.

In this study we collected and investigated water and sediment samples from aquatic environments of surface and subterranean habitats hosting several subterranean environment-adapted organisms. Examined particles were counted and characterized by size, color and shape via visual identification under a microscope, with and without UV light. Furthermore, spectroscopic analyses were carried out in order to identify microplastics typology. Microplastics were found in all examined habitats. In water, microplastics concentration ranged from 37 to 86 items/L, in sediments from 776 to 2064 items/kg. Fibre-shape was the main present, followed by fragments and beads, suggesting multiple sources of pollution, especially textile products. Most of the particles were fluorescent under UV light and were mainly transparent, while not-fluorescent ones were especially black, blue or brown. Samples contained especially polyesters and copolymers. These results highlight intense MP pollution in karst areas, with significant impacts on water quality, and potential effects on subterranean environment-dwelling species. We stress the importance of monitoring pollution in these critical environments for biodiversity and habitat conservation: monitoring in karst areas must become a priority for habitat and species protection, and water resources management, improving analyses on a larger number of aquatic surface and subterranean habitats.

1. Introduction

Microplastics (MPs) are generally defined as the plastics with a 5 mm - 1 μm dimension (Frias and Nash, 2019; International Organization for Standardization, European Committee for Standardization, 2020). MPs can already be industrially synthesized with a small dimension (primary MPs), or can result from the degradation of larger plastics by biological, chemical or physical activities (secondary MPs).

Plastic has been increasingly used in the last decades, however, its resistance, high durability, and low weight make these materials extremely problematic pollutants in natural environments. The small size of MPs increases their dangerousness (Lusher et al., 2015) and

---

* Corresponding author. Department of Environment, Land and Infrastructure Engineering, Politecnico di Torino, Italy.
E-mail address: valentina.balestra@polito.it (V. Balestra).

https://doi.org/10.1016/j.jenvman.2024.120672
Received 11 December 2023; Received in revised form 9 February 2024; Accepted 12 March 2024

makes them easily to transport (Allen et al., 2019; Liu et al., 2019), representing a worldwide concern and an ecological emergency. MPs are highly bioavailable, can be consumed and inhaled by organisms, or assimilated by trophic transfer from prey species (Assas et al., 2020; Devereux et al., 2021; Jahan et al., 2019; Romeo et al., 2015). Furthermore, they can be sources and vectors for other pollutants, such as pesticides (Wanner, 2021), persistent organic pollutants (POPs) (Koelmans et al., 2013), bisphenol A (BPA) (Cheng et al., 2023), chemicals (Rochman et al., 2013), heavy metals (Li et al., 2019; Selvam et al., 2021; Zhou et al., 2019), or antibiotics (Li et al., 2018).

MPs have been investigated mostly in marine environments (e.g. De Lucia et al., 2018; Gholizadeh and Cera, 2022; Tsang et al., 2017), however, recently, more attention has been paid to terrestrial ones (e.g. Ballent et al., 2016; Bertoldi et al., 2021; Boyle and Örmeci, 2020; Wong et al., 2020), highlighting the presence of MPs even in remote areas (e.g. Ambrosini et al., 2019; Neelavannan et al., 2022; Zhang et al., 2021). Nevertheless, research on MP pollution in underground environments, such as karst areas, is lagging behind.

Karst areas are characterized by carbonate rocks, and form the major cave systems worldwide. Karst habitats have a high environmental value, due to the particular geological and hydrological conditions. The subterranean waters of karst areas constitute some of the major water reserves of terrestrial environments (Moldovan et al., 2020) and host specific complex ecosystems that contribute to the functioning of above-ground landscapes (Canedoli et al., 2022; Dossi et al., 2007). Subterranean karst habitats are considered one of the most fragile natural environments of the world (Kurwadkar et al., 2020), therefore, many of these habitats are prioritized at European level and are currently under protection. Karst systems represent the most sensitive aquatic environment worldwide (Dossi et al., 2007). These habitats host organisms with interesting ecological adaptations, such as stygophiles (aquatic, facultative subterranean environment-dwelling organisms) and stygobionts (aquatic organisms that are obligate cave-dwelling) (Culver and Pipan, 2019; Mammola, 2019). However, the particular biology of these species, and the diversity of the aquatic environment in karst areas (groundwater, ponds, pools, stream and lakes), even if interconnected to each others, pose several challenges to conservation (Pipan et al., 2010). Subterranean waters are usually strongly depleted of trophic resources and local species richness of species communities is low (Barzaghi et al., 2017; Zagmajster et al., 2010). Subterranean aquatic environments do not host complex food webs, which makes predation risk limited for some organisms. Hosted communities are not very resilient and can be easily impacted by the variations of environmental conditions (Hose et al., 2022). However, in spring habitats subterranean and surface water features interplay, leading to the formation of complex environments (Cantonati et al., 2020), in which many species exploit both ecosystems during their life cycle (Barzaghi et al., 2017). Different animals provide ecosystem services in these habitats, especially invertebrate, whose movements helps to remix sediments, oxygen and organic materials (Mermillod-Blondin et al., 2023).

MP research in karst environments is recent; this kind of pollution is still little studied in subterranean environment (Balestra and Bellopede, 2022, 2023; Valentić et al., 2022), and lacking in karst ecotones. MP pollution in karst habitats can be linked to direct and indirect human activities, and is strictly related to the hydrodynamic regime of the aquifer, the geology, the karstification degree and the local meteorological conditions. MP contamination in these environments include litter, wastewater, soil and surface water pollution, and athmospheric deposition. MP presence in precipitation was detected in different areas (e.g. Allen et al., 2019; Liu et al., 2019), and this kind of pollution is strongly related to the soil contamination (Zhou et al., 2021). Into the soil, MPs can be transported, travelling throughout the soil pores and rock fractures, and accumulate 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).

The Classical Karst Region was the first studied karst area (Gunn, 2004), and extends across the border between NE Italy and SW Slovenia. This area is rich in peculiar habitats and species, including stygobionts, such as the olm, *Proteus anguinus* Laurenti, 1768, and various aquatic crustaceans like *Troglocaris planinensis* Birštein, 1948.

The olm is the only troglobiont (i.e. specialsit of underground environments) vertebrate present in Europe and is a completely aquatic species. The olm is endemic to the subterranean water flowing in the southern Slovenia, in the Italian Classical Karst, in western Croatia and in Bosnia and Herzegovina (Sket, 1997) and is a priority species in the EU Habitats Directive (92/43/EEC). The olm is the top predator of the karst aquifers thus it can be particularly sensitive to pollution in these environments, and represents a functionally relevant target for a first assessment of MP pollution impacts on groundwaters (Aljančič, 2019; Manenti et al., 2020). Olm occurrence in the Italian sector of the Classical Karts is confirmed by dozens of observations in different sites, although reported only in the grey literature (Mauri et al., 2018; Stoch, 2017 and references therein). Even if the olm is generally considered a classic example of a strict troglobiont organism, there is growingevidence that they can actively exploit the ecotones of underground environments, such as springs (Manenti et al., 2024).

Waters in the Classical Karst have been an important resource for the economy of this region (Zini et al., 2010), but intense exploitation and urbanization are making the waters reserves a highly vulnerable resource (Zini et al., 2010). Today, different zones of this area are protected, however, still even protected ecosystems are close to urban and industrial areas which could threat habitats and species.

The particular subterranean and surface habitats of the Classical Karst Region, the presence of stygobionts in the aquatic environments, and the water reserves, make these environments extremely interesting from an ecological point of view, and very susceptible to environmental changes and pollution, due to the open nature of the system (Balestra et al., 2023; Gillieson, 2011; Kurwadkar et al., 2020; Ruggieri et al., 2017; White, 1988). Therefore, monitoring these habitats is fundamental, especially with regard to the presence of invisible pollutants, such as MPs, which can damage habitats and species, and contaminate waters. Evaluating MPs occurrence in subterranean and surface waters can provide important insights for water management, allowing to combine in situ pollution data and key species conservation.

In this study we investigated water and submerged sediment samples from aquatic subterranean and surface (spring) environments of the Italian sector of the Classical Karst Region, in areas differently exposed to anthropogenic factors and hosting diverse animal communities. The aim of this study are: i) to investigate, for the first time, the presence of MPs in highly vulnerable subterranean and surface aquatic environment of the Italian sector of the Classical Karst Region, ii) to examine MP pollution in waters and submerged sediments of aquatic karst environments hosting the protected *Proteus anguinus*, and iii) to discuss MP abundance and characteristics, potential risks and ecological effects that this kind of pollution could lead to these protected and susceptible habitats and species. This work wants to be the first assessment of MP pollution in these special environments, encouraging more researchers in this field with the aims of promoting appropriate conservation measures for these habitats.

2. Materials and method

2.1. Study area

The Classical Karst Region (Kras) is a rocky limestone plateau of about 900 km^2 located between the NE part of Italy, along the eastern border of Friuli Venezia Giulia region, (Fig. 1), and the SW part of Slovenia, which run for about 15–20 km wide and 40 km long in the SW-NW direction (Visintin and Cucchi, 2010). The name "Classical Karst" comes from the first karst phenomena studies made in this area, from

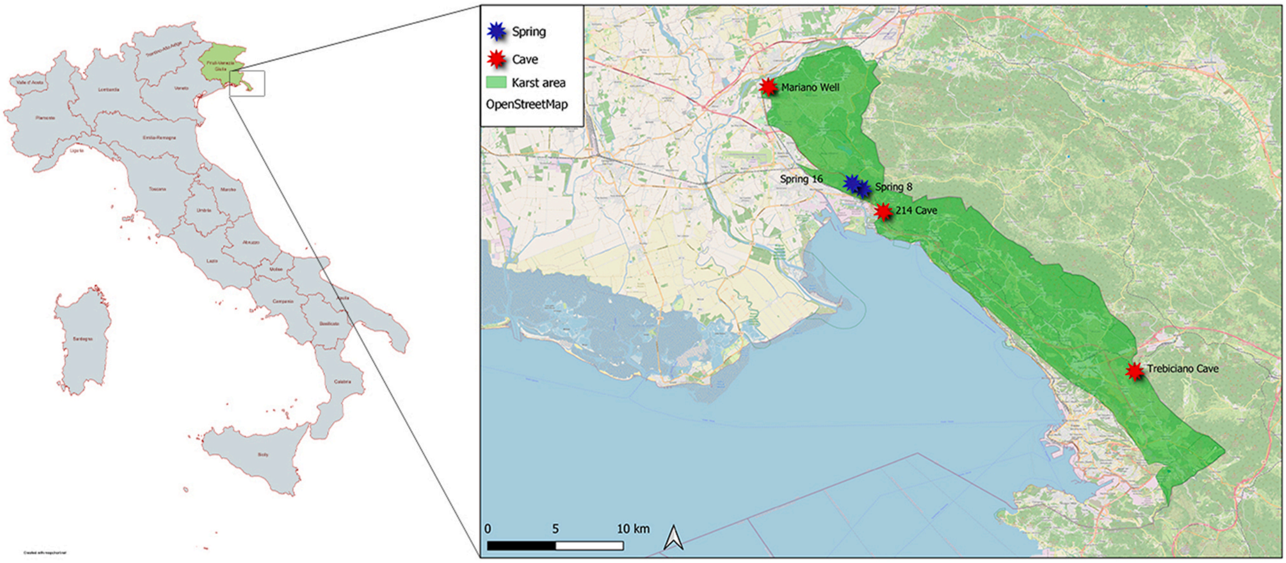

Fig. 1. Study area and sampling points in the Italian sector of Classical Karst, Friuli-Venezia-Giulia Region, Italy. Karst area in bright green, monitored springs in blue stars and monitored caves in red stars (Italy map created with mapchart.net, detail of Italian Classical Karst created with QGIS Desktop 3.12.1 with GRASS 7.8.2 using OpenStreetMap map, modified - openstreetmap.org/copyright). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

which the “karst” term derives (Gunn, 2004). The plateau is mainly composed by Cretaceous to Eocene carbonate rocks (Comeno/Komen Unit) (Cucchi et al., 1987; Placer, 1981) This area is intensely karstified, characterized by a large number of surface karst morphotypes and caves. In the Italian sector, more than 3500 caves and 80 big sinkholes are known (Zini et al., 2010). The major aquifers occurring in the Italian section are connected with Isonzo, Vipacco and Timavo rivers, and with local subterranean waters. The Timavo River flows underground for >70–80 km, with frequent changes of direction in the preferential flows; only two days of travel time occur under high water level conditions (Zini et al., 2010).

Different habitats are present in the Italian sector, of which many are priority habitats according to the European Union classification, such as caves, limestone and scree floors, cliffs, karst lakes and the aquatic and riparian vegetation near the Timavo River. The Timavo River springs are one of the highest-discharge regions in the Mediterranean area (medium discharge of 40 m^3/s, maximum of 175 m^3/s) (Zini et al., 2010). The springs form two large karst depressions, partially filled by Doberdò and Pietrarossa Lakes, a Regional Nature Reserve, one of the few examples of karst pond lake in Europe, separated by a limestone ridge with numerous surface karst phenomena. Animal and plant biodiversity is accentuated by the presence of different natural environments. Karst system of this area host different stygobiont species, such as *Proteus anguinus* and various aquatic crustaceans such as *Troglocaris planinensis*.

Thanks to the high water quality and the abundance of the resources, the Classical Karst waters played an important role for the economy and social development of this region (Zini et al., 2010). This area was strongly altered by human activities, which irreversibly tampered the hydrology of the system (Fornasir, 1929; Gemiti, 2004; Marocco and Melis, 2009). Recently, urbanization, commercial and industrial activities increased too, making the waters reserves a highly vulnerable resource (Zini et al., 2010). Today, different zones of this area are protected, however, the ecosystem is open and close to urban and industrial areas, railways and highways, which could threat habitats and species.

2.2. *Field sampling*

Three subterranean (caves: Mariano Well, Trebiciano Cave and 214 Cave) and two surface (springs: Spring 8 and Spring 16) aquatic environments were selected in the Italian sector of the Classical Karst Region, based on the presence of stygobiont species previously reported in bibliography (Mauri et al., 2018), in particular *Proteus anguinus*, their geographical and biological relevance. Preliminary investigations in the sampling areas confirmed the presence of stygobiont species (Manenti et al., 2024).

Monitoring was conducted in May 2022, at the beginning of the lean period. The subterranean sites are representative of the major aquifers occurring in the Italian sector of the Classical Karst Region; their access requires the use of caving equipment. Surface sites are small springs that flow from the ground, creating small pools. In extreme and fragile environments, such as not touristic caves, it is not always possible to collect large volumes of samples for analysis, due to lack of material, and the difficulty in collecting and/or transporting samples. Moreover, it is necessary to damage these environments as little as possible. Hence, only small amounts of samples were collected in some areas. However, these environments are poorly studied, therefore, any kind of sampling and analysis is crucial to better understand the status of these ecosystems and for their conservation.

214 Cave (Pozzo presso S. Giovanni di Duino, 214/226VG) is located in Duino Aurisina municipality at about 50 m a.s.l. It is a pit with a vertical drop of 47 m, with a water basin on the bottom, strictly connected with the nearby resurgences. This cave is known especially for the exhaustive studies done on the underground course of the Timavo River. A series of tests with dye were previously carried out to examine the arrivals in the resurgences: in flood periods, water arrived in the resurgences after 40 days, highlighting a marginal water table compared to the water courses feeding the springs. The bottom of the pit is closed by a bank of gravel. The cave is adjacent to railway tracks, and is very close to the provincial road and the highway.

Trebiciano Cave (Grotta di Trebiciano, 3/17VG) is located in Trieste municipality, and develops for 1198 m, with a vertical drop of 329 m. The cave entrance is located at about 342 m a.s.l. in a wooded area. It is the best known cave among those existing in the Italian sector of the Classical Karst, both for the underground water flowing in its terminal part and the in-depth studies carried out in different periods. It played an important role in the Timavo River course investigations, being this cave the only intermediate point where the water that disappears in the San Canziano Cave, Slovenia, reappears, albeit for a short distance. From 1974, considerable economic resources and working time were dedicated to preserve and enhance the cavity, and an interesting underground laboratory was set up. Trebiciano cave is not considered a show cave and there are no guided excursions, however, it is an equipped cave, often visited by a lot of speleologists and researchers.

Mariano Well (Pozzo primo di Castelvecchio, 8186/6611VG) is located in the Sagrado municipality (Gorizia karst area). It develops for about 40 m, with a vertical drop of about 23 m, 15 m for reaching the surface of water. The cave entrance, at about 28 m a.s.l., is artificial and opens into the cellars of a building, as it used to collect water for drinking purposes. The original metal structures that went down into the water were demolished and removed in the past, due to their extreme degradation and dangerousness. Underwater explorations did not reveal obvious continuations for speleo-divers.

Spring 8 and 16 are permanent springs identified by preliminary investigations as emitters of a complex aquifer network which connects multiple surface habitats (including two karst lakes) of the Doberdò and Pietrarossa Regional Nature Reserve and also, partially, the subterranean sites sampled (Cucchi et al., 2008).

2.2.1. *Zoological sampling*

In the chosen sites, the occurrence of vertebrate and invertebrate aquatic predators and detritivores was visually recorded, distinguishing between species considered as strictly stygobionts and those known to live in surface waters. Stygobiont species recognition was based on already existing reports for the groundwater fauna of the study area (Stoch, 2017).

Samples of zooplankton and macro-zoobenthos were made too. Plankton collection was performed by filtering 10 L of water from each sampling site through standardized amount of clean cotton cloth. The organisms captured within the fabric were then released into 50 ml of filtered water and then stored in a fridge until being analysed under light microscopy. The detection and quantification of zooplankton was done using light microscopy with 100× magnification, while larger magnification was used to determine the taxon of the organisms. Analyses were performed on 5 ml of the already filtered and resuspended samples, which corresponds to 1 L of the original site water before filtering. Even if this method does not allow an exact quantification of zooplankton species, it provides good insight into relative differences between sites. Macrozoobenthos was sampled by dip-netting within a square of 20 × 20 cm that was positioned marked out at the substrate level. Collected animals were then identified into groups, counted, weighted for biomass, and subsequently released.

2.2.2. *Microplastic sampling*

Sediment and water bulk samples (Hidalgo-Ruz et al., 2012) were collected using pre-cleaned glass jars and nitrile gloves. In the subterranean sites, the jars were limited and wrapped with anti-impact material to transport them safely in the speleological bags. For each sampling area, a quantity between 500 and 1150 mL of surface water and a minimum of 250 g of superficial submerged sediments (upper 5 cm) were collected depending on the water and sediment pool availability (Supplementary table 1). Sediment samples were collected with a metal spoon, cleaned every time with ethanol. Samples were stored in the fridge at 6 °C until laboratory analysis. Two samples (water of Spring 8 and sediment of Spring 16) were damaged during transportation, therefore, they were not analysed.

2.3. *Laboratory analysis*

Plastic equipment was replaced with glass and metal lab equipment, wherever possible. During all steps, nitrile gloves and cotton coats were used by researcher and all open glass jars and equipment were covered with an aluminum foil. All working surfaces and laboratory materials were cleaned with ethanol and milliQ water to avoid MP contamination. All the analyses were carried out under a hood. Blank controls on milliQ water, H_2O_2 30% (Merck), ethanol absolute (VWR Chemicals), and NaCl solution (Carlo Erba NaCl + milliQ water) were done to determine possible contamination during laboratory analysis. The blank correction method used estimates the results of unknown samples by subtracting the contribution of the blank (Shruti and Kutralam-Muniasamy, 2023 and references therein). Where the microplastic characteristics of blanks did not match those of collected samples, the data were reported without subtraction (Edo et al., 2020; Shruti and Kutralam-Muniasamy, 2023).

Water samples were analysed according to the method described in Balestra et al. (2023), improved for these samples, visually rich in organic materials. After being poured in glass beakers, samples were pre-treated through the application of 1:1 30% H_2O_2 solution, covered with an aluminum foil, and left to react for seven days under natural conditions. Each water sample was filtered by a vacuum pump through a 1.2-μm pore size glass fiber filter (Phenomenex, Ø 47 mm). Finally, filters were placed on glass petri dishes, covered with aluminum foil and dried in an oven at 40 °C until completely dry.

Sediment samples were analysed according to the methodology described in Balestra and Bellopede (2023). Sediments were placed in an aluminum box, covered with aluminum foil, and dried into an oven at 40 °C to constant weight. Dried sediments were pre-treated through the application of 1:1 30% H_2O_2 solution, left to react for seven days under natural conditions, and dried again at 40 °C to constant weight. In relation to the quantity of dry sediments, trying to use as much material as possible, three sub-samples of 15 g were selected for each sample via coning and quartering, and put into beakers with 150 ml NaCl solution (200 g NaCl/0.6 L, $\rho = 1.2$). The mixture was stirred with a magnetic mixer for 2 min and settled for 24 h. Subsequently, the supernatant was extracted with a glass pipet and filtered by a vacuum pump through a 1.2-μm pore size glass microfiber filter (Phenomenex, Ø 47 mm). Filters were placed on glass petri dishes, covered with an aluminum foil and dried in an oven at 40 °C until completely dry.

2.4. *Microplastic identification and characterization*

Different plastics contain Fluorescent Whitening Agents (FWAs) (Qiu et al., 2015), therefore, many MPs can be easily detected under an ultraviolet (UV) light (e.g. Balestra and Bellopede, 2022; Balestra et al., 2023; Ehlers et al., 2020; Giardino et al., 2023; Klein and Fischer, 2019; Qiu et al., 2015). However, different organic and inorganic materials are fluorescent under a UV light and not all plastics contain fluorescent additives, especially dark ones (Balestra and Bellopede, 2022; Balestra et al., 2023). Therefore, preliminary screening analysis on filters have to be done.

Particles on filters were observed under a Leitz ORTHOLUX II POL-MK microscope equipped with a DeltaPix Invenio 12EIII 12 Mpx Camera. Microparticles were counted at 2.5 × magnification, enlarging to 10 × or higher magnifications for MPs identification and characterisation; each filter was observed with and without a UV flashlight (Alonefire SV10 365 nm UV flashlight 5 W). Since the accuracy in visually identifying big particles is more reliable than with little ones (Hidalgo-Ruz et al., 2012; Song et al., 2015), it was established to analyse MPs up to 0.1 mm, as suggested in European Commission (2013). Particles not clearly identifiable as MPs were not taken into consideration. Observed MPs were characterised according to the Standardised size and colour sorting system (SCS) (Crawford and Quinn, 2016).

Particles were verified using a micro-Fourier Transform Infrared Spectroscope (μFTIR) Shimadzu AIM-9000 microscope equipped with a Shimadzu IRTracer-100 spectrophotometer and a Shimadzu ATR with a germanium prism. On each filter, 15% of MPs was analysed. Particles on filters were transferred with a dissecting needle on a silver filter (GVS Life Sciences, Membrane Disk 47 mm, 0.8 μm pore size), and were analysed in a spectral range between 4000 and 700 cm^{-1}, with 40 scansions. Atmosphere corrections were applied on the obtained spectra, subsequently compared with the Shimadzu Lab Solution Library ATR Polymer 2, followed by a visual analysis comparison of characteristic bands in the reference spectrum. Spectra were accepted only with a match degree $\geq$80%, as suggested in Fossi et al. (2017).

3. Results

3.1. Fauna occurrence

Our surveys confirmed that both subterranean and surface monitored sites host *Proteus anguinus* populations together with other species usually considered as strictly subterranean, such as the Crustacean Atyid *Troglocaris planinensis* and the Crustacean Spheromatid *Monolistra racovitzai* Strouhal, 1928 (Fig. 2, Table 1). In Trebiciano Cave we also detect the Italian chub, *Squalius squalus* (Bonaparte, 1837), which is a surface fish. Adult olms were detected in all sampling sites. In 214 Cave, some juveniles were observed too, as well as in Spring 16 with the occurrence of a larva. Plankton density varied between cave and spring sites (Table 2), as well as the variety of macrozoobenthic invertebrates sampled (Table 3).

3.2. Microplastic abundance and size

MPs were found in all water and sediment samples. Considering the value found in the blank samples, the possible contamination linked to the pre-existing material in the chemical products used during laboratory activities turned out to be about 10.2 items/L for water samples and 1.7 items/15g for sediment samples. In water, MP concentration ranged between 47.2 and 96.0 items/L, between 36.9 and 85.6 items/L with correction (Supplementary table 2). In sediments, MP concentration ranged between 888.9 ± 101.8 items/kg and 2177.8 ± 473.0 items/kg (average and standard deviation), between 775.6 and 2064.4 items/kg with correction (Supplementary table 3).

The size distribution of collected MPs indicated that mini-MPs (MPs < 1 mm) are the most abundant (79.6%) (Fig. 3A); MPs from 5 to 1 mm accounted for 20.4%. Five mesoplastics (5–25 mm) were found too: two in Spring 16, two in Trebiciano Cave and one in Mariano Well. Mariano Well data indicated percentages very different from other sampling points: about 10.5% of particles had a dimension >1 mm, about half of Spring 16 and one third of Trebiciano and 214 Caves.

Size average percentages of MPs in sediment samples appeared more homogeneous: MPs from 5 to 1 mm accounted for 13.6%, and MPs <1 mm for 86.4%. Again, Mariano Well showed different percentages, though only slightly: about 17.1% of particles had a dimension >1 mm, otherwise, the other sampling areas were between 12.2 and 14.0%. No mesoplastics were found in sediment samples.

3.3. Microplastic shape

Fibre-shape dominated all water samples (64.8%), followed by fragments (30.0%), beads (3.2%), film (1.6%) and foam (0.4%) (Figs. 3C and 4). The values found for the waters of Spring 16, 214 Cave and Trebiciano Cave were similar, with high percentage of fibres (>73%), followed by fragments (18–23%). Other shapes had percentages less than 4%. Mariano Well sample had very different values, with fibers and fragments around 45%, and 7.4% of beads. Foam shape was found only in Mariano Well.

Fibre-shape dominated all sediment samples (60.6%), followed by fragments (23.7%), beads (14.8%) and film (0.8%) (Figs. 3D and 4). No foam was found. The values found for the sediments of Spring 8, 214 Cave and Mariano Well were similar, with high percentages of fibers (75.0–80.5%), followed by fragments (14.0–22.5%). Other shapes had percentages less than 3.5%. Instead, Trebiciano Cave sample had very different values, with fibers, fragments and beads respectively 35.7, 32.7 and 31.6%.

3.4. Microplastic fluorescence and colour

Figs. 4 and 5 underline the importance of visual identification under microscope, in order to analyse non-fluorescent particles (about 35% in waters and 31% in sediments).

The fluorescent particle abundance percentages in water (Fig. 5A) were similar for each sampling area, with a slight increase in Trebiciano Cave (70.8%) and a minimum value in 214 Cave (60.8%). The major part of fluorescent particles had blue fluorescence (68.7%), followed by green (17.8%) and red one (6.7%) (Fig. 5C). A 6.7% of particles had other fluorescence colours. Spring 16 and Mariano Well MPs had very

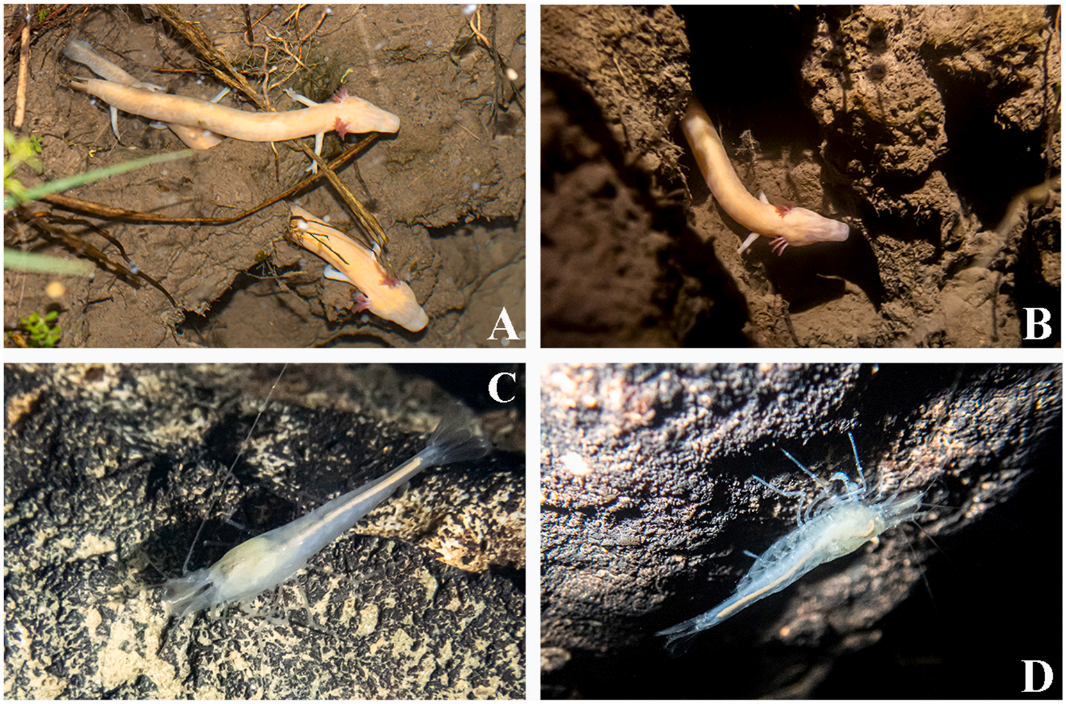

Fig. 2. Stygobiont fauna in surface and subterranean monitored environments. A: *Proteus anguinus* in surface habitat (spring); B: *Proteus anguinus* in subterranean habitat (cave); C: *Troglocaris planinensis* in surface habitat (spring); D: *Troglocaris planinensis* in subterranean habitat (cave) (photos: V. Balestra).

Table 1
Results of visual encounter surveys on cave-dwelling and surficial predators and detritivores in both caves and springs.

Site name	Environment	Stygobiont predators	Stygobiont detritivores	Surficial predators	Surficial detritivores
Spring 8	surface	*Proteus anguinus*	*Troglocaris planinensis*	*Dendrocoelum lacteum*	*Asellus aquaticus* *Emmericia patula*
Spring 16	surface	*Proteus anguinus*	*Monolistra racovitzai*	*Esox lucius* *Polycelis nigra*	*Asellus aquaticus* *Emmericia patula*
214 Cave	subterranean	*Proteus anguinus*	*Troglocaris planinensis*	-	–
Trebiciano Cave	subterranean	*Proteus anguinus*	*Troglocaris planinensis*	*Squalius squalus*	–
Mariano Well	subterranean	*Proteus anguinus*	*Troglocaris planinensis*	-	–

Table 2
Results of plankton samplings in both caves and springs.

Site name	Ciliata (micro)	Gastrotricha (micro)	Rotifera (meso)	Hydrachnida (meso)	Ostracoda (meso)	Nauplius (meso)	Copepoda (macro)	N. micro	N. meso	N. macro
Spring 8	0	0	0	0	2	6	3	0	8	3
Spring 16	657	0	0	0	0	0	1	657	0	1
214 Cave	0	0	0	0	0	0	0	0	0	0
Trebiciano Cave	0	0	0	0	0	0	0	0	0	0
Mariano Well	0	0	0	0	0	0	0	0	0	0

Table 3
Results of dip-netting samplings.

Site name	N. squares sampled	Taxa and density (in parenthese)	Total weight (g)
Spring 8	1	*Polycelis nigra* (30), Gammaridae (10), Lumbricidae (1), *Emmericia patula* (6)	0.69
Spring 16	2	*Asellus aquaticus* (4), *Planorbis* sp. (2), *Planorbarius* sp. (1), Gammaridae (2)	0.12
214 Cave	10	*Troglocaris planinensis* (50)	24.2
Trebiciano Cave	10	*Troglocaris planinensis* (10), *Dendrocoelum* sp. (1)	5.19
Mariano Well	3	None	0

similar fluorescence colour percentages, Trebiciano Cave particles had only blue, green and red fluorescence, instead, 214 Cave had higher blue fluorescent particles (77.4%), low values of green fluorescent MPs (12.9%), and no red fluorescent ones. (Fig. 5C).

The fluorescent particle abundance percentages in sediments (Fig. 5B) were similar for 214 Cave and Mariano Well (>80%), while Spring 8 and Trebiciano Cave had about 60% of fluorescent MPs. The major part of fluoresce particles had blue fluorescence (74.2%), followed by red (8.6%) and green (6.1%) one (Fig. 5D). A 11% of particles had other fluorescent colours. MPs in Spring 8, 214 Cave and Mariano Well had similar fluorescence colours: more than 80% of fluorescent particles had blue fluorescence. Instead, Trebiciano Cave sample returned very different percentages: blue fluorescent particles were only 58.6%, and high values of different colours were present (24.1%) (Fig. 5D).

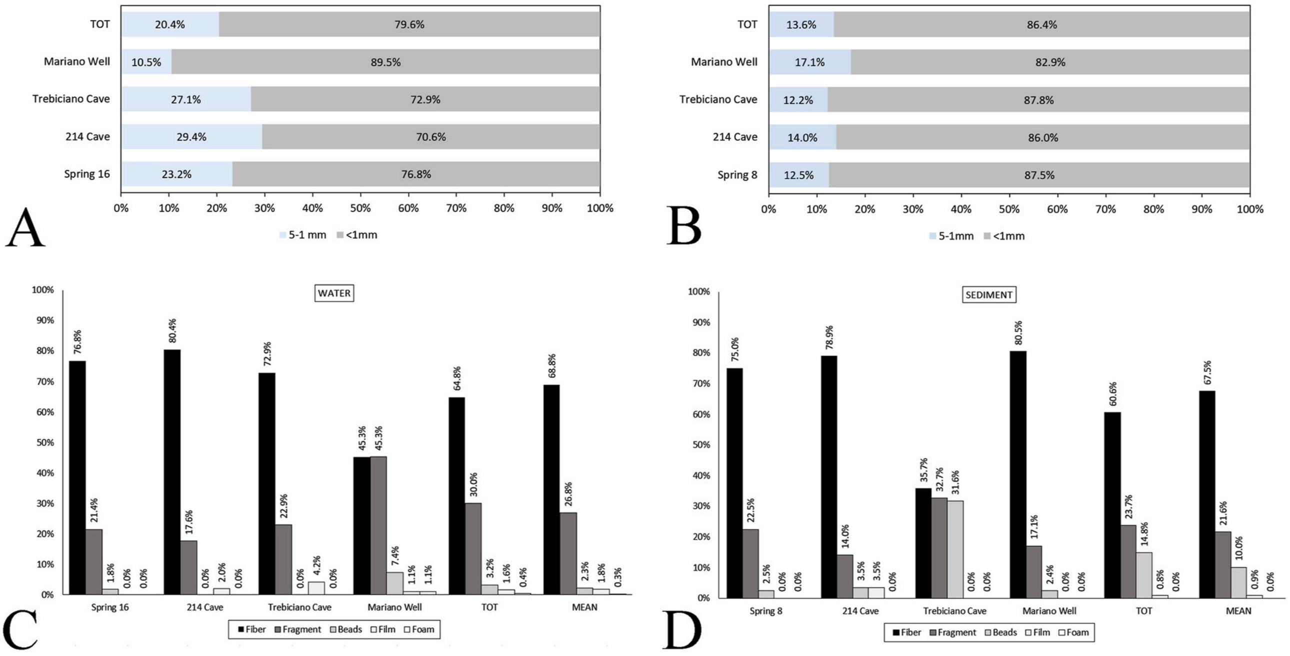

Fig. 3. Microplastic characterisation in water and sediments of spring and cave aquatic environments of the Italian Classical Karst. A: Microplastic size in water samples; B: Microplastic size in sediment samples; C: Microplastic shape in water samples; D: Microplastic shape in sediment samples; (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

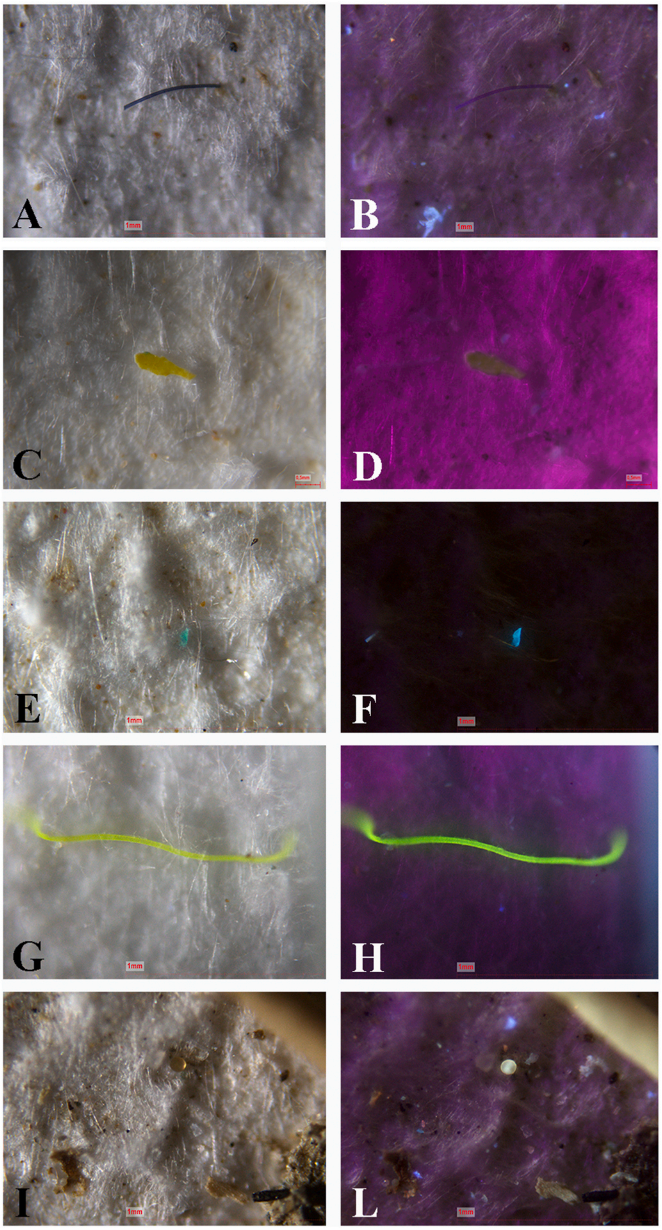

Fig. 4. Images of microplastics found in the Italian Classical Karst water environments under microscope, with and without UV light. A, B: blue fibre without fluorescence; C, D: yellow fragment without fluorescence; E, F: green fragment with blue fluorescence; G, H: yellow fibre with yellow fluorescence; I, L: amber sphere (microbead) with white fluorescence (Photos: V. Balestra). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Of the fluorescent MPs found in water samples, 57.4% were transparent, followed by green (10.5%), and beige (5.6%) ones; particles with other colours had percentages between 0.6 and 4.9% (Fig. 5E). Non-fluorescent MPs were mainly black (48.3%), blue (23.0%), red (5.7%), and transparent (5.7%); particles with other colours had percentages between 1.1 and 4.6% (Fig. 5G).

Of the fluorescent MPs found in sediments, 63.8% were transparent, followed by blue (7.4%), amber (7.4%) and beige ones (6.1%); particles with other colours had percentages between 0.6 and 3.7% (Fig. 5F). Non-fluorescent MPs were mainly black (56.2%), brown (15.1%), blue (8.2%), and grey (5.5%); particles with other colours have percentages between 1.4 and 2.7% (Fig. 5H).

Analyzing data by sampling area, it is possible to observe that most common particles were transparent and black. However, some differences were present considering the other colours: percentages for each color were very different from each other, not all colours were present in each sampling area, the two springs had no brown MPs, and Spring 8 had less colours than the other sampling areas.

3.5. *Characterisation of microplastic by μFTIR-ATR*

Water samples contained especially polyesters (15%) and copolymers (10%), followed by PVAc (polyvinyl acetate), PET (polyethylene terephthalate), PTFE (polytetrafluoroethylene), PAM (polyacrylamide), EVOH (ethylene vinyl alcohol), and epoxy resin (Fig. 6A). The 45% of particle spectra obtained during the μFTIR-ATR analysis on water samples had a match below 80%.

In sediments samples were found especially copolymers (25%), followed by PA (polyamide), EVOH, PAM, polyester, POM (polyoxymethylene or polyacetal), CSPE (polyethylene chlorosulfonated) (Fig. 6B). Particles spectra obtained during the μFTIR-ATR analysis were all counterable with high matches.MPs found in waters and sediments showed different degradation degree (Fig. 6): MPs spectra in water were much more difficult to examine with spectroscopy because particles were often contaminated by different materials and very degraded.

Examined MPs were randomly selected. Most of the examined MPs were fibers of different size and color. Examined fragment resulted epoxy resin, and were <1 mm and transparent.

4. Discussion

MPs were found in all examined water and sediment samples, highlighting an intense MP pollution in surface and subterranean fresh water karst habitats. Comparisons with other karst areas are challenging since methodologies and analyses are different and can affect the results. Moreover, the size of MPs taken into account, the sampling point, and environmental conditions may vary MP concentrations over time. However, some assumptions on the amounts of MPs in karst environments can be made (Table 4). MPs found in the Classical Karst Region highlighted that MP abundance in surface and subterranean waters is higher than most of underground waters investigated so far (Table 4), with the exception of Bossea Cave (Balestra et al., 2023) waters and ab alluvial aquifer monitored in Australia (Samandra et al., 2022). The size range of MPs detected in these works is different and the size range observed in our study is often wider, therefore, may be detected higher MP concentrations. Moreover, the examined waters are stagnant for long periods, and this can determine an accumulation of pollutants over time. Karst lakes are the surface manifestation of the karst subterranean water discharge (Culver and Pipan, 2014); in the lean period the water level decreases limiting water exchanges and aquatic habitats to channels and pools, creating peculiar environments. Water level in the examined cave areas is subject to seasonal variations, and water resources are limited to pools and large ponds in different periods of the year. Water level variations and connections between surface and subterranean systems in the area are likely to exacerbate the impact of microplastics in karst aquifers, being involved multiple food chains (including the terrestrial one) and habitats.

To our knowledge, very few works examined MP pollution in sediments collected in subterranean karst environments and no one in the aquatic ones, therefore, comparisons are possible only with not aquatic karst environments. In sediments of three NW Italian show caves, were found 3823–4695 MP/kg along the tourist path, and 1600–3186 MP/kg in the speleological areas (MPs from 5 to 0.1 mm) (Balestra and Bellopede, 2023). The MP amount found in Trebiciano Cave was similar to those found in the speleological areas of the Italian show caves, while in 214 Cave, Mariano Well and Spring 8 the abundances were lower. In the Škocjan region, Slovenia, about 6667 items/m^3 were found in Kačna Cave sediments and 60,000 items/m^3 in Škocjan Caves system (Valentić et al., 2022), very low values compared to those found in the Classical

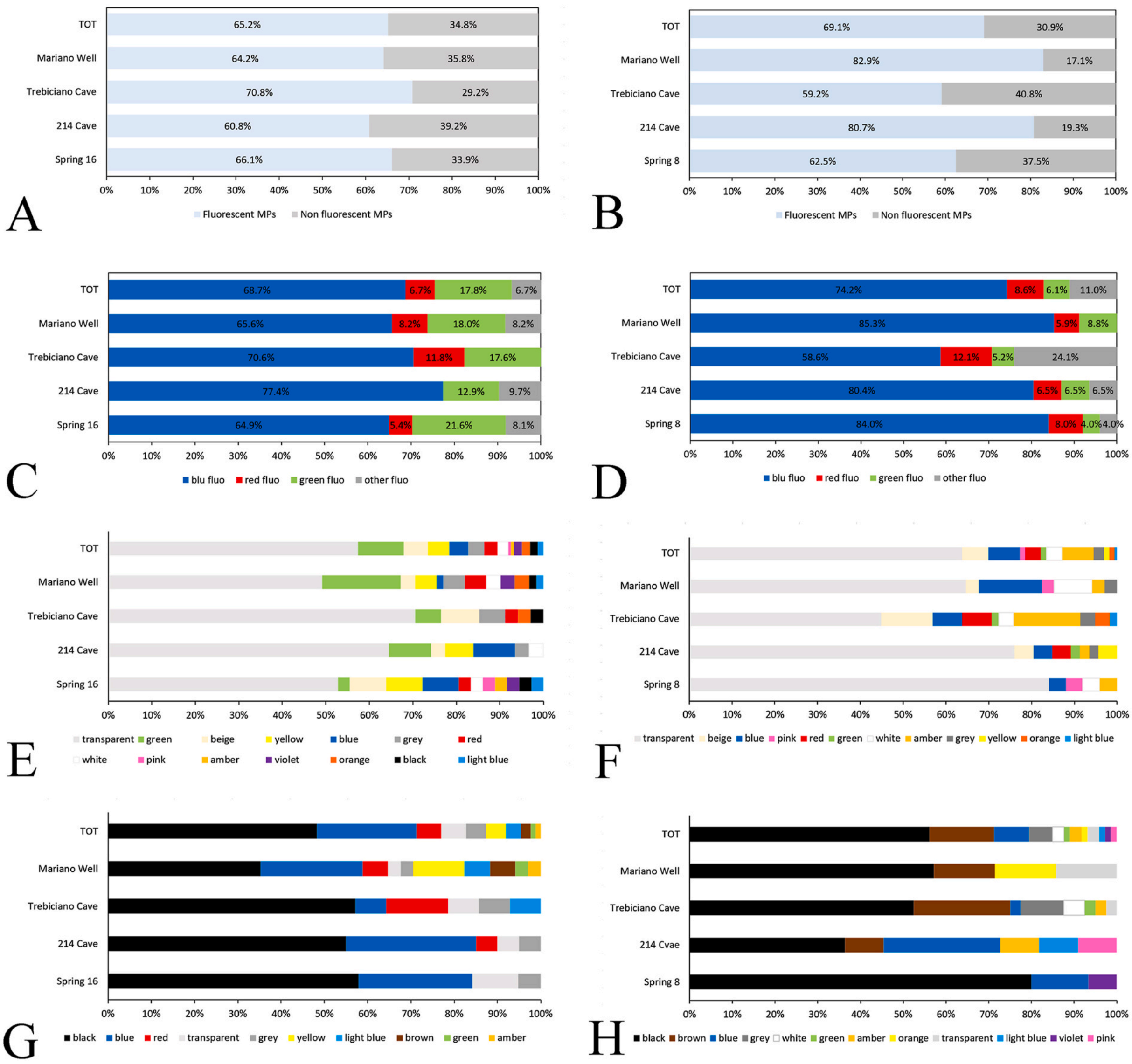

Fig. 5. Fluorescence and colour of examined microplastics. A: Fluorescent microplastic in water samples; B: Fluorescent microplastic in sediment samples; C: Fluorescence colour in water samples; D: Fluorescence colour in sediment samples; E: colours of fluorescent microplastics in water samples; F: colours of fluorescent microplastics in sediment samples; G: colours of non-fluorescent microplastics in water samples; H: colours of non-fluorescent microplastics in sediment samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

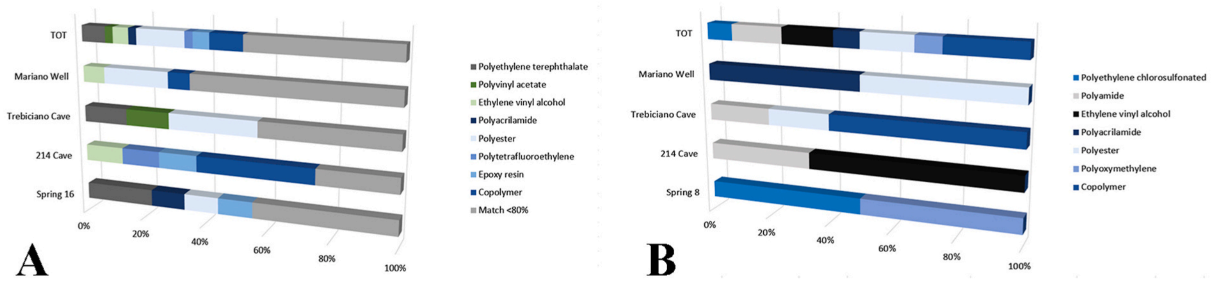

Fig. 6. Typology of examined microplastics. A: typology of microplastics in water samples; B: typology of microplastics in sediment samples.

Karst. Considering caves as conservative environments (Chiarini et al., 2022), even though the water levels may vary during the year, is reasonable to suppose an accumulation of pollutants in sediments of aquatic karst environments over time.

Percentages of MPs from 5 to 1 mm found in the Classical Karst waters were similar to those found in the Bossea Cave system (Italy) which accounted for 17.1% of the total MPs found (Balestra et al., 2023), while they were lower than those found in the waters collected in Guizhou Province, China, which MPs from 5 to 3 mm accounted for 44.20% (An et al., 2022). Percentages of MPs from 5 to 1 mm found in

Table 4
Microplastic concentrations found in previous work.

	Typology	Considered size	MP concentration	References
Bossea Cave system, Italy	Springs	5–0.1 mm	23 and 29 items/L	Balestra et al. (2023)
Timava Springs, Slovenia	Springs	5-1 mm	9.52±<0.01 items/m^3	Valentić et al. (2022)
Springs in Illinois, USA	Springs	5–0.5 mm	Maximum concentration of 15.2 items/L, mean of 7.9 items/L	Panno et al. (2019)
Wells in Illinois, USA	Groundwaters	5–0.5 mm	Maximum concentration of 4.4 items/L, mean of 2.8 items/L)	Panno et al. (2019)
Wells and bore wells in coastal south India	Groundwaters	5–0.1 mm	median concentration of 4.2 items/L, maximum concentration of 10.1 items/L	Selvam et al. (2021)
NW Germany	Groundwaters	5 mm-20 μm	5.6 items/L	Mintenig et al. (2019)
Alluvial unconfined aquifer in Victoria, Australia	Groundwaters	500-20 μm	average concentration of 38 ± 8 items/L	Samandra et al. (2022)
Bossea Cave, Italy	Cave waters	5–0.1 mm	12 to 54 items/L, mean of 28 items/L	Balestra et al. (2023)
Kačna cave, Slovenia	Cave waters	5-1 mm	16.67 ± 0.01 items/m^3	Valentić et al. (2022)
Škocjan Caves system, Slovenia	Cave waters	5-1 mm	9.55 ± 16.64 items/m^3	Valentić et al. (2022)
Postojna Cave, Slovenia	Cave waters	5-1 mm	1.00 ± 1.48 items/m^3	Valentić et al. (2022)

the sediment of the Classical Karst aquatic environments were less than those found in the non-aquatic sediments in the Italian show caves, which accounted for 25.3% in Toirano Caves, 27.5% in Borgio Verezzi Cave, and 27.3% in Bossea Cave (Balestra and Bellopede, 2023).

According to previous studies (e.g. Balestra and Bellopede, 2022, 2023; Balestra et al., 2023; Panno et al., 2019; Valentić et al., 2022), the major part of MPs in the examined karst environments were fibres, both in liquid and solid matrices. About 60% of textile production worldwide is synthetic (Barrows et al., 2018; Boucher and Friot, 2017), suggesting pollution in this area could be linked mainly to synthetic textiles deterioration. Fragments had a relevant percentage too (30.0% in water and 23.7% in sediments). Italian show cave sediments along the tourist paths had a 5.5–12.1% of fragments (Balestra and Bellopede, 2023). In Bossea Cave karst system water, Italy, a 4.3% of fragments were found (Balestra et al., 2023). However, Classical Karst values about fragments were less than those found in the unconfined groundwater aquifer monitored in Victoria, Australia (94%) (Samandra et al., 2022). Fragments could have been produced from waste deterioration in the environment or nearest human activities. The monitored springs are located near important high speed roads, and 214 Cave develops near the railway track, therefore, MPs pollution in this sampling areas could be linked to the transit of vehicules, road markings, road wear, tire, road and railway sub-beds, train and track degradation, dust and garbage (Andersson-Sköld et al., 2020; Burghardt and Pashkevich, 2023). Abundant garbage was found near the train railway, highway and roads close to all examined areas. Mariano Well is located inside an urban complex with concrete constructions for drinking water collection in its initial stretch, and its waters resulted the most polluted by fragments. Therefore, most of the pollution in the well probably comes from the surface anthropogenic activities, which reach the subsoil with the infiltration of water into the ground. Another possible source of pollution could be leak from domestic water pipes or sewerage, that can percolates underground. The inevitable mixing of waste water with rainwater does not allow for any general assessment on the basis of water balances. Therefore, it is difficult to estimate the sewerage losses. Examining the other MP shapes found in this well, it is possibile to observe a high number of microbeads (7.4%) too. Microbeads are often used in care and beauty products (Anagnosti et al., 2021), such as scrubs, toothpaste, and cosmetics, reinforcing the hypothesis of sewerage losses. An interesting value of microbeads was also found in the sediment sample of Trebiciano Cave (31.6%), as well as the highest percentage of fragments (32.7%) among the sediment samples. Trebiciano Cave is an equipped cavity, visited by speleologists and researchers from 1974, therefore, this kind of pollution could be linked to the frequent presence of people, and to the surface human activities and garbage, which could be transported into the cavity by the waters of the inner stream.

As reported in previous research (Balestra and Bellopede, 2022, 2023; Balestra et al., 2023), most of the MPs found were fluorescent under UV light (65% in water and 69% in sediments). These percentages were lower than those found in waters (85%) and sediments (88%) of Bossea Cave, Italy, and in sediments of Borgio Verezzi Cave (74%), Italy. Instead, percentages found in the Classical Karst were higher than that observed in sediments of Toirano Caves, Italy (60%) (Balestra and Bellopede, 2023; Balestra et al., 2023). These data show as the identification of MPs by UV light can be a good method, however, it is not sufficient for all particle analysis. MP fluorescence could provide information on consumption by organisms too. For examples, fluorescent pellets, fibers and fragments of anthropogenic origin were found in stygofauna collected from three groundwater bodies in Italian karst areas (Sforzi et al., 2024). Ecosystem health is heavily affected by MP accumulation in the environment and some organisms wrongly mistake MPs for trophic resources consuming them (Gomiero et al., 2018; Sforzi et al., 2024). Ingestion of MPs induce different deleterious effects on organisms, such as pathological alteration or blockages of digestive processes. MP colour can provide 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), making this step of analysis fundamental for ecological and environmental research. In marine biota, most of MPs particles show a blue colour (32.9%), followed by white (24.7%), black (18.8%) and transparent (16.5%) particles (Ugwu et al., 2021). In fish and marine mammals, the predominant colour of MPs found were blue, black, transparent, and white. Different colours were found in MPs ingested by birds, where transparent colour are the majority (55.6%), followed by blue (33.3%) and brown (11.1%). Some studies identified black plastics as the most often ingested ones by the analysed sea animals (Jahan et al., 2019; Lusher et al., 2013); in Romeo et al. (2015) transparent and white MPs occurred in the stomach of all analysed top sea predators, whereas blue particles and yellow ones were found only in some of them. In Sforzi et al., 2024, some anthropogenic particles of blue colour were found in the digestive tract of crustaceans of karst caves and springs, suggesting the potential presence of MPs in the fecal pellets. In this study, transparent, black and blue particles were found in high amounts in all examined superficial and subterranean sampling areas; white particles were present in all sampling area, even if only in small percentages. Therefore, further investigations could be done in the future to better understand if the aquatic organisms of the Classical Karst, especially stygobionts, consume MPs mistaking them for trophic resources. MP colour and fluorescence could be not of particular interest for stygobiont ingestion, being these animals mostly blind, however, they may be important for consumption by surface organisms and animals less adapted to the hypogeal environment. In addition, MP colour can provide indication about the chemical pollutants which can bind to them by contaminating them (e.g. Frias et al., 2010; Karapanagioti et al., 2011). In marine environments, high levels of pollutants were found on yellow and black particles (Frias et al., 2010; Karapanagioti et al., 2011). Black particles were found in all sampling areas with high amounts, and different yellow MPs were observed in Spring 16, 214 Cave and Mariano Well. Most of the non-fluorescent MPs found in the Classical Karst was black (48.3% in water and 56.2% in sediment), and different yellow particles in fluorescent MPs were found in water samples (4.9%). Future investigations should be carried out to better understand the presence of pollutants related to MPs in those environments.

At the moment, MP typology comparisons with other karst systems are possible only between few areas (An et al., 2022; Balestra and Bellopede, 2023; Balestra et al., 2023; Romano et al., 2023; Valentić et al., 2022): PET, PA, and polyester, have been found frequently, together with PE (polyethylene), PP (polypropylene), and PS (polystyrene), PVA (polyvinyl alcohol), EVOH, PVC (polyvinyl chloride), acrylic adhesive, PU (polyurethane), PAM, EVA (ethylene vinyl acetate), EPDM (ethylene propylene diene rubber), PMMA (polymethylmethacrylate), PBT (polybutylene terephthalate), PVFM (polyvinyl formal), PTFE, copolyester, TPV (thermoplastic vulcanizates) and EBA (ethylene butyl acrylate). Many of these plastics are commonly used in the textile industry, validating the primary hypothesis on the origin of synthetic fibres from the textiles.

Microscopy analysis is one of the most used methods for MP characterization, being economic, however, visual analysis can't provide information on the chemical composition of MPs and is a laborious method (Huang et al., 2023). Moreover, when MP particle size is too small, visual analysis method alone is not recommended (European Commission, 2013; Hidalgo-Ruz et al., 2012; Song et al., 2015). Spectroscopic analyses are useful to confirm particles identified with microscopy, determining their composition, however, they are time-consuming. Moreover, surface particles collected in natural environments are often covered by other materials and impurities, and/or contaminated by other pollutants, consequently, MP spectra are difficult to match with high percentages with spectra libraries (Song et al., 2015). A combination of several methods is the optimal choice to identify MPs in natural environments (Song et al., 2015), therefore, in this work MPs were detected by means of microscopic and spectroscopic techniques.

Pollution is major cause of modification of wildlife habitats, determining the loss of sensitive species. Anthropogenic activities, atmospheric depositions and infiltrations through fractures and soil can lead to MP pollution in karst systems, posing a risk to water quality and biodiversity conservation of these particular ecosystems. From ciliates to detritivores and predators, the direct or indirect consumption of MPs available in the environment has been highlighted (e.g. Devereux et al., 2021; Lusher et al., 2013; Pennati et al., 2022). Our data suggest that both surface and subterranean aquatic species in karst system are potentially exposed to MP pollution. Even in relatively simplified ecosystems like subterranean water, MPs could affect multiple levels of the trophic web, and impact species with important regulatory functions across the ecosystem, such as the olm. Usually, the quantification of contaminants/pollutants affecting apex, long-lived predators can represent an effective method for detecting their transfer through food webs. As the olm is the apex predator in Classical Kast aquifers, evaluating the level of MP occurrence in the habitats where olm occur represents a first step to assess the biological effects on organisms depending from groundwaters and, potentially, also humans. Taking into account previous work on aquatic fauna, it is reasonable to think that stygobionts exposed to MPs could suffer detrimental impacts such as disrupted feedings, compromised reproductivity or organs damage, especially in the gastrointestinal system (Anbumani and Kakkar, 2018; Zhang et al., 2023). MPs in stygobionts digestive tract and gut were recently detected in cave and spring habitats (Sforzi et al., 2024), but biological response to MP exposure in this habitats is unknow. Further studies assessing MPs content in the stomachs of apex predators such as the olm will be useful to elucidate MPs impacts in aquatic subterranean and surface environments. Groundwater and spring fauna plays an important role in the sediment transport and the cycle of nutrient, positively contributing to the overall health of the aquatic ecosystem (Mermillod-Blondin et al., 2023). MPs along food chains could have implications until higher trophic levels, disrupting the above-mentioned processes, with cascading ecological consequences (Zhang et al., 2023). MPs occurring in subterranean environments may impact also the boundaries of subterranean environments, including fragile ecotones like springs.

Microplastic monitoring is essential to define the health status of the environment, and, consequently, to establish strategies. Ecological connections between habitats have to be taken into account, such as species displacements between surface and subterranean environments. Habitats are frequently subjected to partial conservation measures which rarely take into account the connections between ecosystems and the movement of species between them. These connections must be considered when planning comprehensive measure of protection. Assessing and promoting changes at the political and social levels that encourage real plastic reduction strategies, and provide adequate education following the environmental sustainability principles are needed for the management and conservation of water resources, protected habitats and species. Microplastic pollution monitoring in karst areas must become a priority for the habitat and species conservation and the water resources management, implementing analyses on a larger number of aquatic surface and subterranean habitats.

5. Conclusion

These first results improve knowledge on MP pollution in natural environments, drawing attention on karst water quality, and the potential risks that could affect to protected habitats and species. This first investigation in the Italian sector of the Classical Karst, hosting different protected and stygobiont species, documents the presence of microplastics in surface and subterranean waters, drawing attention on the potential exposition of stygobiont species (but not only) to microplastic pollution, which could attain multiple levels of the trophic resources. The concentration of MPs in monitored waters and sediments was highly variable, with most of particles being transparent and fluorescent under UV light. Vice versa, not-fluorescent particles were mainly black or dark. Fibre-shape dominated the samples, suggesting that synthetic textiles are the main source of microplastic pollution in this area. Other possible sources of pollution could be linked to nearby human activities, providing useful references for further research.

Microplastic monitoring is a key step to understand the health status of environment and to establish management strategies. Future studies are needed to better understand MP sources and transport in karst areas, verifying the potential effects on ecosystems and aquatic organisms. Long-term monitoring, considering more subterranean and surficial sites, will help understanding the impact of MP pollution on habitats. Monitoring small MPs, pollutants linked to MPs, and other micropollutants in karst aquatic environments could provide important information on the health of the karst systems and the possible damage that these pollutants could cause even to tiny organisms. Potentially negative consequences from MP pollution for subterranean water safety at all the levels, such as ecological functionality, biodiversity distribution, ecosystem services and human health should be investigated too.

Surface and underground environments in karst areas are closely connected, therefore, greater efforts should be made to establish more comprehensive measure of protection. Microplastic pollution monitoring in karst areas must become a priority for the habitat and species conservation and the water resources management, implementing analyses on a larger number of aquatic surface and subterranean habitats. A combination of awareness, willingness and new ideas will allow us to curtail the threat that pollution poses to wildlife and other species, including humans.

Funding

This work was realized with internal funds, with the support of the Multimodal Analysis Laboratory (LAM) of DIATI – Politecnico di Torino, of DESP – Università degli Studi di Milano, and with the collaboration of Biologia Sotterranea Piemonte – Gruppo di Ricerca.

CRediT authorship contribution statement

Valentina Balestra: conceptualisation, methodology, validation, formal analysis, investigation, resources, data curation, writing – original draft, writing – review and editing, visualisation, project administration. **Matteo Galbiati:** investigation. **Stefano Lapadula:** investigation, writing – review and editing. **Veronica Zampieri:** investigation, writing – review and editing. **Filippomaria Cassarino:** investigation. **Magdalena Gajdošová:** investigation, writing – review and editing. **Benedetta Barzaghi:** investigation, resources. **Raoul Manenti:** investigation, resources, writing – original draft, writing – review and editing, funding acquisition. **Gentile Francesco Ficetola:** resources, writing – review and editing, funding acquisition. **Rossana Bellopede:** methodology, validation, resources, writing – review and editing, project administration, funding acquisition.

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.

Data availability

Data will be made available on request.

Acknowledgments

The authors are grateful to Edgardo Mauri of the Trieste Speleovivarium “Erwin Pichl” and Società Adriatica di Speleologia for helping us during sampling, and Claudio De Regibus of Politecnico di Torino for helping with laboratory equipment.

Research on *P. anguinus* was authorized by the “Ministero della Transizione Ecologica”, permit number 003267 of March 15, 2022; research on subterranean invertebrates was authorized by Regione Friuli Venezia Giulia, permit number 0019599 of march 21 2022.

This article has been possible thanks to some information and insights learned during the activities carried out by COST Action CA20101 Plastics monitoRIng detectiOn RemedIaTion recoverY - PRIORITY, supported by COST (European Cooperation in Science and Technology). www.cost.eu.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.120672.

References

Aljančič, G., 2019. History of research on Proteus anguinus Laurenti 1768 in Slovenia/Zgodovina raziskovanja človeške ribice (Proteus anguinus Laurenti 1768) v Sloveniji. Folia biologica et geologica 60, 39–69. https://doi.org/10.3986/fbg0050.

Allen, S., Allen, D., Phoenix, V.R., Le Roux, G., Durántez Jiménez, P., Simonneau, A., Binet, S., Galop, D., 2019. Atmospheric transport and deposition of microplastics in a remote mountain catchment. Nat. Geosci. 12, 339–344. https://doi.org/10.1038/s41561-019-0335-5.

Ambrosini, R., Azzoni, R.S., Pittino, F., Diolaiuti, G., Franzetti, A., Parolini, M., 2019. First evidence of microplastic contamination in the supraglacial debris of an alpine glacier. Environ. Pollut. 253, 297–301. https://doi.org/10.1016/j.envpol.2019.07.005.

An, X., Li, W., Lan, J., Adnan, M., 2022. Preliminary study on the distribution, source, and ecological risk of Typical microplastics in karst groundwater in Guizhou Province, China. Int. J. Environ. Res. Publ. Health 19, 14751. https://doi.org/10.3390/ijerph192214751.

Anagnosti, L., Varvaresou, A., Pavlou, P., Protopapa, E., Carayanni, V., 2021. Worldwide actions against plastic pollution from microbeads and microplastics in cosmetics focusing on European policies. Has the issue been handled effectively? Mar. Pollut. Bull. 162, 111883 https://doi.org/10.1016/j.marpolbul.2020.111883.

Anbumani, S., Kakkar, P., 2018. Ecotoxicological effects of microplastics on biota: a review. Environ. Sci. Pollut. Res. 25, 14373–14396. https://doi.org/10.1007/s11356-018-1999-x.

Andersson-Sköld, Y., Johannesson, M., Gustafsson, M., Järlskog, I., Lithner, D., Polukarova, M., Strömvall, A.-M., 2020. Microplastics from Tyre and Road Wear: a Literature Review.

Assas, M., Qiu, X., Chen, K., Ogawa, H., Xu, H., Shimasaki, Y., Oshima, Y., 2020. Bioaccumulation and reproductive effects of fluorescent microplastics in medaka fish. Mar. Pollut. Bull. 158, 111446 https://doi.org/10.1016/j.marpolbul.2020.111446.

Balestra, V., Bellopede, R., 2022. Microplastic pollution in show cave sediments: first evidence and detection technique. Environ. Pollut. 292, 118261 https://doi.org/10.1016/j.envpol.2021.118261.

Balestra, V., Bellopede, R., 2023. Microplastics in caves: a new threat in the most famous geo-heritage in the world. Analysis and comparison of Italian show caves deposits. J. Environ. Manage. 342, 118189 https://doi.org/10.1016/j.jenvman.2023.118189.

Balestra, V., Vigna, B., De Costanzo, S., Bellopede, R., 2023. Preliminary investigations of microplastic pollution in karst systems, from surface watercourses to cave waters. J. Contam. Hydrol. 252, 104117 https://doi.org/10.1016/j.jconhyd.2022.104117.

Ballent, A., Corcoran, P.L., Madden, O., Helm, P.A., Longstaffe, F.J., 2016. Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments. Mar. Pollut. Bull. 110, 385–395. https://doi.org/10.1016/j.marpolbul.2016.06.037.

Barrows, A., Cathey, S.E., Petersen, C.W., 2018. Marine environment microfiber contamination: global patterns and the diversity of microparticle origins. Environ. Pollut. 237, 275–284. https://doi.org/10.1016/j.envpol.2018.02.062.

Barzaghi, B., Ficetola, G.F., Pennati, R., Manenti, R., 2017. Biphasic predators provide biomass subsidies in small freshwater habitats: a case study of spring and cave pools. Freshw. Biol. 62, 1637–1644. https://doi.org/10.1111/fwb.12975.

Bertoldi, C., Lara, L.Z., Fernanda, A.d.L., Martins, F.C., Battisti, M.A., Hinrichs, R., Fernandes, A.N., 2021. First evidence of microplastic contamination in the freshwater of lake Guaíba, Porto Alegre, Brazil. Sci. Total Environ. 759, 143503 https://doi.org/10.1016/j.scitotenv.2020.143503.

Boucher, J., Friot, D., 2017. Primary Microplastics in the Oceans: a Global Evaluation of Sources. IUCN, p. 43. https://doi.org/10.2305/IUCN.CH.2017.01.en.

Boyle, K., Örmeci, B., 2020. Microplastics and nanoplastics in the freshwater and terrestrial environment: a review. Water 12, 2633. https://doi.org/10.3390/w12092633.

Burghardt, T.E., Pashkevich, A., 2023. Road markings and microplastics–A critical literature review. Transport. Res. Transport Environ. 119, 103740 https://doi.org/10.1016/j.trd.2023.103740.

Canedoli, C., Ficetola, G.F., Corengia, D., Tognini, P., Ferrario, A., Padoa-Schioppa, E., 2022. Integrating landscape ecology and the assessment of ecosystem services in the study of karst areas. Landsc. Ecol. 1–19 https://doi.org/10.1007/s10980-021-01351-2.

Cantonati, M., Stevens, L.E., Segadelli, S., Springer, A.E., Goldscheider, N., Celico, F., Filippini, M., Ogata, K., Gargini, A., 2020. Ecohydrogeology: the interdisciplinary convergence needed to improve the study and stewardship of springs and other groundwater-dependent habitats, biota, and ecosystems. Ecol. Indicat. 110, 105803 https://doi.org/10.1016/j.ecolind.2019.105803.

Carpenter, E.J., Anderson, S.J., Harvey, G.R., Miklas, H.P., Peck, B.B., 1972. Polystyrene spherules in coastal waters. Science 178, 749–750. https://doi.org/10.1126/science.178.4062.749.

Cheng, Z., Lin, X., Wu, M., Lu, G., Hao, Y., Mo, C., Li, Q., Wu, J., Wu, J., Hu, B.X., 2023. Combined effects of polyamide microplastics and Hydrochemical factors on the transport of bisphenol A in groundwater. Separations 10, 123. https://doi.org/10.3390/separations10020123.

Chia, R.W., Lee, J.-Y., Kim, H., Jang, J., 2021. Microplastic pollution in soil and groundwater: a review. Environ. Chem. Lett. 1–14 https://doi.org/10.1007/s10311-021-01297-6.

Chiarini, V., Duckeck, J., De Waele, J., 2022. A global Perspective on sustainable show cave tourism. Geoheritage 14, 1–27. https://doi.org/10.1007/s12371-022-00717-5.

Crawford, C.B., Quinn, B., 2016. Microplastic Pollutants. Elsevier, Amsterdam.

Cucchi, F., Franceschini, G., Zini, L., 2008. Hydrogeochemical investigations and groundwater provinces of the Friuli Venezia Giulia Plain aquifers, northeastern Italy. Environ. Geol. 55, 985–999. https://doi.org/10.1007/s00254-007-1048-4.

Cucchi, F., Radrizzani, C.P., Pugliese, N., 1987. The Carbonate Stratigraphic Sequence of the Karst of Trieste (Italy).

Culver, D.C., Pipan, T., 2014. Shallow Subterranean Habitats: Ecology, Evolution, and Conservation. Oxford University Press, USA.

Culver, D.C., Pipan, T., 2019. The Biology of Caves and Other Subterranean Habitats. Oxford University Press, USA.

De Lucia, G.A., Vianello, A., Camedda, A., Vani, D., Tomassetti, P., Coppa, S., Palazzo, L., Amici, M., Romanelli, G., Zampetti, G., 2018. Sea water contamination in the vicinity of the Italian minor islands caused by microplastic pollution. Water 10, 1108. https://doi.org/10.3390/w10081108.

Devereux, R., Hartl, M.G., Bell, M., Capper, A., 2021. The abundance of microplastics in cnidaria and ctenophora in the North Sea. Mar. Pollut. Bull. 173, 112992 https://doi.org/10.1016/j.marpolbul.2021.112992.

Dossi, C., Ciceri, E., Giussani, B., Pozzi, A., Galgaro, A., Viero, A., Viganò, A., 2007. Water and snow chemistry of main ions and trace elements in the karst system of Monte Pelmo massif (Dolomites, Eastern Alps, Italy). Mar. Freshw. Res. 58, 649–656. https://doi.org/10.1071/MF06170.

Edo, C., González-Pleiter, M., Tamayo-Belda, M., Ortega-Ojeda, F.E., Leganés, F., Fernández-Piñas, F., Rosal, R., 2020. Microplastics in sediments of artificially recharged lagoons: case study in a Biosphere Reserve. Sci. Total Environ. 729, 138824 https://doi.org/10.1016/j.scitotenv.2020.138824.

Ehlers, S.M., Maxein, J., Koop, J.H., 2020. Low-cost microplastic visualization in feeding experiments using an ultraviolet light-emitting flashlight. Ecol. Res. 35, 265–273. https://doi.org/10.1111/1440-1703.12080.

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, p. 126. https://doi.org/10.2788/99475.

Fahrenfeld, N., Arbuckle-Keil, G., Beni, N.N., Bartelt-Hunt, S.L., 2019. Source tracking microplastics in the freshwater environment. ChemistryTrAC Trends Anal. Chem. 112, 248–254. https://doi.org/10.1016/j.trac.2018.11.030.

Fornasir, D., 1929. Relazione al progetto esecutivo della Bonifica del Lisert. Consorzio di Bonifica del Lisert-Monfalcone, Trieste, pp. 67–77.

Fossi, M.C., Romeo, T., Baini, M., Panti, C., Marsili, L., Campani, T., Canese, S., Galgani, F., Druon, J.-N., Airoldi, S., 2017. Plastic debris occurrence, convergence areas and fin whales feeding ground in the Mediterranean marine protected area Pelagos sanctuary: a modeling approach. Front. Mar. Sci. 167 https://doi.org/10.3389/fmars.2017.00167.

Frei, S., Piehl, S., Gilfedder, B., Löder, M.G., Krutzke, J., Wilhelm, L., Laforsch, C., 2019. Occurrence of microplastics in the hyporheic zone of rivers. Sci. Rep. 9, 1–11. https://doi.org/10.1038/s41598-019-51741-5.

Frias, J., Sobral, P., Ferreira, A.M., 2010. Organic pollutants in microplastics from two beaches of the Portuguese coast. Mar. Pollut. Bull. 60, 1988–1992. https://doi.org/10.1016/j.marpolbul.2010.07.030.

Frias, J.P., Nash, R., 2019. Microplastics: Finding a consensus on the definition. Mar. Pollut. Bull. 138, 145–147. https://doi.org/10.1016/j.marpolbul.2018.11.022.

Gemiti, F., 2004. Le sorgenti Sardos e l'approvvigionamento idrico della provincia di Trieste. Atti e Memorie della Commissione Grotte "E. Boegan 39, 67–80.

Gholizadeh, M., Cera, A., 2022. Microplastic contamination in the sediments of Qarasu estuary in Gorgan Bay, south-east of Caspian sea, Iran. Sci. Total Environ., 155913 https://doi.org/10.1016/j.scitotenv.2022.155913.

Giardino, M., Balestra, V., Janner, D., Bellopede, R., 2023. Automated method for routine microplastic detection and quantification. Sci. Total Environ. 859, 160036 https://doi.org/10.1016/j.scitotenv.2022.160036.

Gillieson, D.S., 2011. Management of Caves, Karst Management. Springer, pp. 141–158.

Gomiero, A., Strafella, P., Fabi, G., 2018. From Macroplastic to Microplastic Litter: Occurrence, Composition, Source Identification and Interaction with Aquatic Organisms. Experiences from the Adriatic Sea, Plastics in the Environment. IntechOpen.

Gunn, J., 2004. Encyclopedia of Caves and Karst Science. Taylor & Francis.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060–3075. https://doi.org/10.1021/es2031505.

Hose, G.C., Chariton, A., Daam, M.A., Di Lorenzo, T., Galassi, D.M.P., Halse, S.A., Reboleira, A.S., Robertson, A.L., Schmidt, S.I., Korbel, K.L., 2022. Invertebrate traits, diversity and the vulnerability of groundwater ecosystems. Funct. Ecol. 36, 2200–2214. https://doi.org/10.1111/1365-2435.14125.

Huang, Z., Hu, B., Wang, H., 2023. Analytical methods for microplastics in the environment: a review. Environ. Chem. Lett. 21, 383–401. https://doi.org/10.1007/s10311-022-01525-7.

International Organization for Standardization, European Committee for Standardization, 2020. Plastics - Environmental Aspects - State of Knowledge and Methodologies. CEN ISO/TR 21960:2020).

Jahan, S., Strezov, V., Weldekidan, H., Kumar, R., Kan, T., Sarkodie, S.A., He, J., Dastjerdi, B., Wilson, S.P., 2019. Interrelationship of microplastic pollution in sediments and oysters in a seaport environment of the eastern coast of Australia. Sci. Total Environ. 695, 133924 https://doi.org/10.1016/j.scitotenv.2019.133924.

Karapanagioti, H., Endo, S., Ogata, Y., Takada, H., 2011. Diffuse pollution by persistent organic pollutants as measured in plastic pellets sampled from various beaches in Greece. Mar. Pollut. Bull. 62, 312–317. https://doi.org/10.1016/j.marpolbul.2010.10.009.

Klein, M., Fischer, E.K., 2019. Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany. Sci. Total Environ. 685, 96–103. https://doi.org/10.1016/j.scitotenv.2019.05.405.

Koelmans, A.A., Besseling, E., Wegner, A., Foekema, E.M., 2013. Plastic as a carrier of POPs to aquatic organisms: a model analysis. Environ. Sci. Technol. 47, 7812–7820. https://doi.org/10.1021/es401169n.

Kurwadkar, S., Kanel, S.R., Nakarmi, A., 2020. Groundwater pollution: occurrence, detection, and remediation of organic and inorganic pollutants. Water Environ. Res. 92, 1659–1668. https://doi.org/10.1002/wer.1415.

Li, J., Zhang, K., Zhang, H., 2018. Adsorption of antibiotics on microplastics. Environ. Pollut. 237, 460–467. https://doi.org/10.1016/j.envpol.2018.02.050.

Li, X., Mei, Q., Chen, L., Zhang, H., Dong, B., Dai, X., He, C., Zhou, J., 2019. Enhancement in adsorption potential of microplastics in sewage sludge for metal pollutants after the wastewater treatment process. Water Res. 157, 228–237. https://doi.org/10.1016/j.watres.2019.03.069.

Liu, K., Wang, X., Fang, T., Xu, P., Zhu, L., Li, D., 2019. Source and potential risk assessment of suspended atmospheric microplastics in. Shanghai. Sci. Total Environ. 675, 462–471. https://doi.org/10.1016/j.scitotenv.2019.04.110.

Lusher, A.L., Hernandez-Milian, G., O'Brien, J., Berrow, S., O'Connor, I., Officer, R., 2015. Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean: the True's beaked whale Mesoplodon mirus. Environ. Pollut. 199, 185–191. https://doi.org/10.1016/j.envpol.2015.01.023.

Lusher, A.L., Mchugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67, 94–99. https://doi.org/10.1016/j.marpolbul.2012.11.028.

Lwanga, E.H., Gertsen, H., Gooren, H., Peters, P., Salánki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2017. Incorporation of microplastics from litter into burrows of Lumbricus terrestris. Environ. Pollut. 220, 523–531. https://doi.org/10.1016/j.envpol.2016.09.096.

Mammola, S., 2019. Finding answers in the dark: caves as models in ecology fifty years after Poulson and White. Ecography 42, 1331–1351. https://doi.org/10.1111/ecog.03905.

Manenti, R., Di Nicola, M.R., Zampieri, V., Grassi, G., Creanza, T., Mauri, E., Ficetola, G. F., Barzaghi, B., 2024. Wandering outside of the Styx: surface activity of an iconic subterranean vertebrate, the olm (*Proteus anguinus*). Ecology. https://doi.org/10.1002/ecy.4252.

Manenti, R., Melotto, A., Guillaume, O., Ficetola, G.F., Lunghi, E., 2020. Switching from mesopredator to apex predator: how do responses vary in amphibians adapted to cave living? Behav. Ecol. Sociobiol. 74, 1–13. https://doi.org/10.1007/s00265-020-02909-x.

Marocco, R., Melis, R., 2009. Stratigrafia e paleogeografia del "lacus timavi"(Friuli Venezia Giulia). Alp. Mediterr. Quat. 22, 157–170.

Mauri, E., Abbona, I., Papi, F., 2018. Storia delle ricerche del Proteo in Italia e attuali conoscenze a 250 anni dalla sua prima descrizione da parte di Laurenti. In: Giulia, R. A.F.V. (Ed.), Speleo 2018. Conference, Trieste, pp. 121–125.

McGechan, M., 2002. SW—soil and water: transport of particulate and colloid-sorbed contaminants through soil, part 2: trapping processes and soil pore geometry. Biosyst. Eng. 83, 387–395. https://10.1006/bioe.2002.013.

Mermillod-Blondin, F., Hose, G.C., Simon, K.S., Korbel, K., Avramov, M., Vander Vorste, R., 2023. Role of Invertebrates in Groundwater Ecosystem Processes and Services, Groundwater Ecology and Evolution. Elsevier, pp. 263–281.

Mintenig, S., Löder, M., Primpke, S., Gerdts, G., 2019. Low numbers of microplastics detected in drinking water from ground water sources. Sci. Total Environ. 648, 631–635. https://doi.org/10.1016/j.scitotenv.2018.08.178.

Moldovan, O.T., Bercea, S., Năstase-Bucur, R., Constantin, S., Kenesz, M., Mirea, I.C., Petculescu, A., Robu, M., Arghir, R.A., 2020. Management of water bodies in show caves–a microbial approach. Tour. Manag. 78, 104037 https://doi.org/10.1016/j.tourman.2019.104037.

Neelavannan, K., Sen, I.S., Lone, A.M., Gopinath, K., 2022. Microplastics in the high-altitude Himalayas: assessment of microplastic contamination in freshwater lake sediments, Northwest Himalaya (India). Chemosphere 290, 133354. https://doi.org/10.1016/j.chemosphere.2021.133354.

Panno, S.V., Kelly, W.R., Scott, J., Zheng, W., McNeish, R.E., Holm, N., Hoellein, T.J., Baranski, E.L., 2019. Microplastic contamination in karst groundwater systems. Groundwater 57, 189–196. https://doi.org/10.1111/gwat.12862.

Pennati, R., Castelletti, C., Parolini, M., Scarì, G., Mercurio, S., 2022. Mixotrophic flagellate ingestion boosts microplastic accumulation in ascidians. J. Exp. Zool. Part A: Ecological and Integrative Physiology 337, 639–644. https://doi.org/10.1002/jez.2596.

Pipan, T., Holt, N., Culver, D.C., 2010. How to protect a diverse, poorly known, inaccessible fauna: identification and protection of source and sink habitats in the epikarst. Aquat. Conserv. Mar. Freshw. Ecosyst. 20, 748–755. https://doi.org/10.1002/aqc.1148.

Placer, L., 1981. Geologic structure of southwestern Slovenia. Geologija 24, 27–60.

Qiu, Q., Peng, J., Yu, X., Chen, F., Wang, J., Dong, F., 2015. Occurrence of microplastics in the coastal marine environment: first observation on sediment of China. Mar. Pollut. Bull. 98, 274–280. https://doi.org/10.1016/j.marpolbul.2015.07.028.

Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 1–7. https://doi.org/10.1038/srep03263.

Romano, E., Bergamin, L., Di Bella, L., Baini, M., Berto, D., D'Ambrosi, A., Di Fazio, M., Galli, M., Medeghini, L., Panti, C., Provenzani, C., Rampazzo, F., Fossi, M.C., 2023. First record of microplastic in the environmental matrices of a Mediterranean marine cave (Bue Marino, Sardinia, Italy). Mar. Pollut. Bull. 186, 114452 https://doi.org/10.1016/j.marpolbul.2022.114452.

Romeo, T., Pietro, B., Pedà, C., Consoli, P., Andaloro, F., Fossi, M.C., 2015. First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. Mar. Pollut. Bull. 95, 358–361. https://doi.org/10.1016/j.marpolbul.2015.04.048.

Ruggieri, R., Forti, P., Antoci, M.L., De Waele, J., 2017. Accidental contamination during hydrocarbon exploitation and the rapid transfer of heavy-mineral fines through an overlying highly karstified aquifer (Paradiso Spring, SE Sicily). J. Hydrol. 546, 123–132. https://doi.org/10.1016/j.jhydrol.2016.12.046.

Samandra, S., Johnston, J.M., Jaeger, J.E., Symons, B., Xie, S., Currell, M., Ellis, A.V., Clarke, B.O., 2022. Microplastic contamination of an unconfined groundwater aquifer in Victoria, Australia. Sci. Total Environ. 802, 149727 https://doi.org/10.1016/j.scitotenv.2021.149727.

Selvam, S., Jesuraja, K., Venkatramanan, S., Roy, P.D., 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. J. Hazard Mater. 402, 123786 https://doi.org/10.1016/j.jhazmat.2020.123786.

Sforzi, L., Agostina, T.D.C., Di Lorenzo, T., Galassi, D.M.P., Balestra, V., Piccini, L., Cabigliera, S.B., Ciattini, S., Laurati, M., Chelazzi, D., Martellini, T., Cincinelli, A., 2024. (Micro-)plastics in Saturated and Unsaturated Groundwater Bodies: First Evidence of Presence in Groundwater Fauna and Habitats. Sustainability.

Shruti, V., Kutralam-Muniasamy, G., 2023. Blanks and bias in microplastic research: implications for future quality assurance. Trends in Environmental Analytical Chemistry, e00203. https://doi.org/10.1016/j.teac.2023.e00203.

Sket, B., 1997. Distribution of Proteus (Amphibia: Urodela: Proteidae) and its possible explanation. J. Biogeogr. 24, 263–280. https://doi.org/10.1046/j.1365-2699.1997.00103.x.

Song, Y.K., Hong, S.H., Jang, M., Han, G.M., Rani, M., Lee, J., Shim, W.J., 2015. A comparison of microscopic and spectroscopic identification methods for analysis of microplastics in environmental samples. Mar. Pollut. Bull. 93, 202–209. https://doi.org/10.1016/j.marpolbul.2015.01.015.

Stoch, F., 2017. Il Lacus Timavi: la fauna acquatica sotterranea, con particolare riguardo alle risorgive del Fiume Timavo. Atti e Memorie della Commissione Grotte "E. Boegan 47, 173–203.

Tsang, Y.Y., Mak, C.W., Liebich, C., Lam, S.W., Sze, E.T., Chan, K.M., 2017. Microplastic pollution in the marine waters and sediments of Hong Kong. Mar. Pollut. Bull. 115, 20–28. https://doi.org/10.1016/j.marpolbul.2016.11.003.

Ugwu, K., Herrera, A., Gómez, M., 2021. Microplastics in marine biota: a review. Mar. Pollut. Bull. 169, 112540 https://doi.org/10.1016/j.marpolbul.2021.112540.

Valentić, L., Kozel, P., Pipan, T., 2022. Microplastic pollution in vulnerable karst environments: case study from the Slovenian classical karst region. Acta Carsol. 51, 79–92. https://doi.org/10.3986/ac.v51i1.10597.

Viaroli, S., Lancia, M., Re, V., 2022. Microplastics contamination of groundwater: Current evidence and future perspectives. A review. Sci. Total Environ., 153851 https://doi.org/10.1016/j.scitotenv.2022.153851.

Visintin, L., Cucchi, F., 2010. The Classical Karst. The Classical Karst, pp. 1000–1007.

Wanner, P., 2021. Plastic in agricultural soils–a global risk for groundwater systems and drinking water supplies?–a review. Chemosphere 264, 128453. https://doi.org/10.1016/j.chemosphere.2020.128453.

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., Yap, P.-S., 2020. Microplastics in the freshwater and terrestrial environments: Prevalence, fates, impacts and sustainable solutions. Sci. Total Environ. 719, 137512 https://doi.org/10.1016/j.scitotenv.2020.137512.

Zagmajster, M., Culver, D.C., Christman, M.C., Sket, B., 2010. Evaluating the sampling bias in pattern of subterranean species richness: combining approaches. Biodivers. Conserv. 19, 3035–3048. https://doi.org/10.1007/s10531-010-9873-2.

Zhang, S., Wu, H., Hou, J., 2023. Progress on the effects of microplastics on aquatic Crustaceans: a review. Int. J. Mol. Sci. 24, 5523. https://doi.org/10.3390/ijms24065523.

Zhang, Y., Gao, T., Kang, S., Allen, S., Luo, X., Allen, D., 2021. Microplastics in glaciers of the Tibetan Plateau: evidence for the long-range transport of microplastics. Sci. Total Environ. 758, 143634 https://doi.org/10.1016/j.scitotenv.2020.143634.

Zhou, Y., He, G., Jiang, X., Yao, L., Ouyang, L., Liu, X., Liu, W., Liu, Y., 2021. Microplastic contamination is ubiquitous in riparian soils and strongly related to elevation, precipitation and population density. J. Hazard Mater. 411, 125178 https://doi.org/10.1016/j.jhazmat.2021.125178.

Zhou, Y., Liu, X., Wang, J., 2019. Characterization of microplastics and the association of heavy metals with microplastics in suburban soil of central China. Sci. Total Environ. 694, 133798 https://doi.org/10.1016/j.scitotenv.2019.133798.

Zini, L., Visintin, L., Peric, B., Cucchi, F., Gabrovsek, F., 2010. Transboundary Aquifers: Challenges and New Directions: Characterisation of a Transboundary Karst Aquifer: the Classical Karst, Transboundary Aquifers/ISARM 2010 International Conference, pp. 6–8.