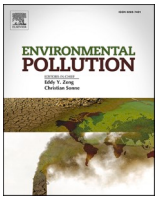
Microplastic pollution in show cave sediments: First evidence and detection technique

Environmental Pollution 292 (2022) 118261

Contents lists available at ScienceDirect

Environmental Pollution

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



Microplastic pollution in show cave sediments: First evidence and detection technique☆

Valentina Balestra *, Rossana Bellopede

Department of Environment, Land and Infrastructure Engineering, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129, Torino, Italy

ARTICLE INFO

Keywords:
Microplastic
Tourist cave
Plastic contamination
Environmental pollution
Karst system

ABSTRACT

Microplastic particles are a global problem, which has been widely found in marine and terrestrial environments. However, microplastic pollution in caves and karst aquifers is still poorly studied. To improve the current knowledge of microplastic pollution, we investigated the sediments of a show cave in Italy. We developed a methodology based on a cave-adapted version of the methods used in several studies to detect microplastics from sediments of different environments and with various laboratory tests. The microplastics were extracted from sediments via density separation and subjected to organic matter removal. Filters were observed with and without UV light under a microscope, before and after organic matter removal, and the microplastics were characterised according to shape, colour, and size, with visual identification. About 55% of the fibres observed under the microscope on filters were removed via organic matter removal. An average of 4390 items/kg dry weight was calculated for the touristic zone and 1600 items/kg dry weight for the speleological/research section. Fibre (84.9%) was the most abundant shape, and most microplastics were smaller than 1 mm, accounting for 85.4%, of which 58.4% were shorter than 0.5 mm. The highest microplastic abundance was fluorescent under UV light (87.7%); however, 12.3% of the microplastics observed on filters were not fluorescent. Most fluorescent fibres were transparent (84%), whereas blue (46.1%) and black (22.4%) fibres were more common for the non-fluorescent ones. Our results highlight the presence of microplastics in show caves, and we provide a valid non-invasive and non-expensive analytical technique for the preparation and isolation of microplastics from cave sediments, giving useful information for evaluating the environmental risks posed by microplastics in show caves.

1. Introduction

Microplastics (MPs) (plastics < 5 mm) are a heterogeneous group of solid polymers containing particles that originate from primary production or from the degradation of larger plastic materials (secondary production) (Corami et al., 2020; Henry and Klepp, 2018; Prata et al., 2019; Zhou et al., 2019). The largest contribution is constituted by the shedding of synthetic clothes in washing machines (35%), followed by tire wear (28%) and urban dust (24%) (Corami et al., 2020). Microplastic pollution is a global issue: contamination has been found in marine and terrestrial environments (e.g. Jiang et al., 2019; Panno et al., 2019; Phuong et al., 2018; Vianello et al., 2013), and several organisms can ingest MPs directly or indirectly (Corami et al., 2020; Henry and Klepp, 2018; Wright et al., 2013). In addition, MPs can adsorb persistent organic pollutants, which can be transferred to animal tissues after ingestion (Panno et al., 2019) and can be vectors for heavy metals (Zhou et al., 2019). Different studies have demonstrated MP contamination not only in populated areas (e.g. Jahan et al., 2019; Zhou et al., 2019), but also in remote areas such as glaciers, mountains, Antarctica, or forests, being them extremely mobile (e.g. Ambrosini et al., 2019; Cabrera et al., 2020; Cincinelli et al., 2017; Van Cauwenberghe et al., 2013; Zhang et al., 2021). However, MP pollution in caves and karst aquifers is still largely unknown (e.g. Panno et al., 2019).

Karst caves are extreme and fragile ecosystems with an exceptional scientific value, rich in endemic and troglobitic fauna and in speleothems, representing an extraordinary and unique Quaternary archive (Cigna and Forti, 2013). Groundwater in karst aquifers constitutes about 25% of the global drinking water sources (Panno et al., 2019). Over the past decades, the interest in the underground karst environments and its natural wonders has grown remarkably, not only from the scientific

☆ This paper has been recommended for acceptance by Eddy Y. Zeng.

* Corresponding author.
E-mail addresses: valentina.balestra@polito.it (V. Balestra), rossana.bellopede@polito.it (R. Bellopede).

https://doi.org/10.1016/j.envpol.2021.118261
Received 18 June 2021; Received in revised form 27 September 2021; Accepted 28 September 2021
Available online 30 September 2021

viewpoint, but also from an economic perspective. Cavities transformed into show caves may be easily damaged, making it important to follow strict rules before, during and after development to maintain the aesthetic and scientific values of the caves (Cigna and Forti, 2013). Tourist caves are the most attractive natural features of geotourism, allowing more than 50,000 visitors/year in the major show caves of the world (Cigna, 2016; Cigna and Forti, 2013). However, human presence in these caves has led to several environmental issues such as pollution, cave climate change, corrosion of speleothems, lampenflora and variations in cave species abundance and distribution. Visiting the cave, tourists transport dust and lint (organic and synthetic fibres from clothing) into caves, covering and damaging speleothems and walls and providing an artificial food source for different species (Baker and Roberts, 2015; Chelius et al., 2009; Christman, 2019; Jablonsky et al., 1993; Pate, 1999). Lint can damage speleothems indirectly by providing nutrients for acid-producing organisms that can dissolve limestone (Jablonsky et al., 1993) and can be incorporated into the cave formation growth. Some lint fibre analyses gave a synthetic fibre content between 30 and 75% (Christman, 2019; Jablonsky et al., 1993). Moreover, only few works on lint in caves have been done (e.g. Chelius et al., 2009; Christman, 2019; Jablonsky et al., 1993), and the potential impact of MP fibres has not been explored.

Since there are several methods to analyse and quantify MPs in different environments, there is no established standard for evaluating them, even if a technical report has been recently published to harmonise procedures and measures (International Organization for Standardization and European Committee for Standardization, 2020). Many existing methods are expensive and require the use of specific instruments. Nevertheless, monitoring concentrations of MPs in the environment is essential to better understand their sources, where they can be transported and the impacts they have on ecosystems (Prata et al., 2019). Moreover, MP monitoring is necessary to understand potential risks for humans (Henry and Klepp, 2018).

Assessing MP contamination in caves is crucial for different reasons: they can be ingested by cave animals, endanger the fragile ecosystems of the caves, irreversibly damage speleothems and paleontological or archaeological findings depositing on them and pollute karst aquifers. The aims of this study are: i) to summarise a valid non-invasive and non-expensive analytical technique for the separation of MPs from cave sediments and their identification, highlighting the importance of organic matter removal, and ii) to investigate and discuss, for the first time, the abundance, shape and morphological characteristic of MPs in show cave sediments.

2. Materials and methods

2.1. Field sampling and data collection

Bossea Cave is located in Frabosa Soprana municipality, Piedmont, SW Italian Alps (Fig. 1), at 836 m a.s.l., and is a protected nature reserve established by Piedmont Region in 2011. It is the first show cave of Italy, opened to the public in 1874, and receives about 12,000 tourists/year.

The cavity is the terminal sector of a large karst system developing in the Maudagna-Corsaglia watershed, between the Prato Nevoso dell and the Corsaglia River. The cavity has a single entrance and a development of about 2800 m, with an ascending structure. It develops in a tectonic contact between the Middle Triassic Dolomie di San Pietro dei Monti formation (dolostone and carbonate rocks) and permotriassic metavolcanics (Antonellini et al., 2019). The cavity is crossed by a subterranean river with a flow rate ranging from 50 to 1200 l/s, which directly flows in the Corsaglia River. Bear (*Ursus spelaeus*) bones and scratch marks were found in different parts of the cave, and in the "Bear room", it is possible to see some paleontological finds.

Two underground karst laboratories to study hydrogeology, hypogean meteorology, radon activity and subterranean biology are located in the touristic and non-touristic parts of the Bossea Cave, managed by

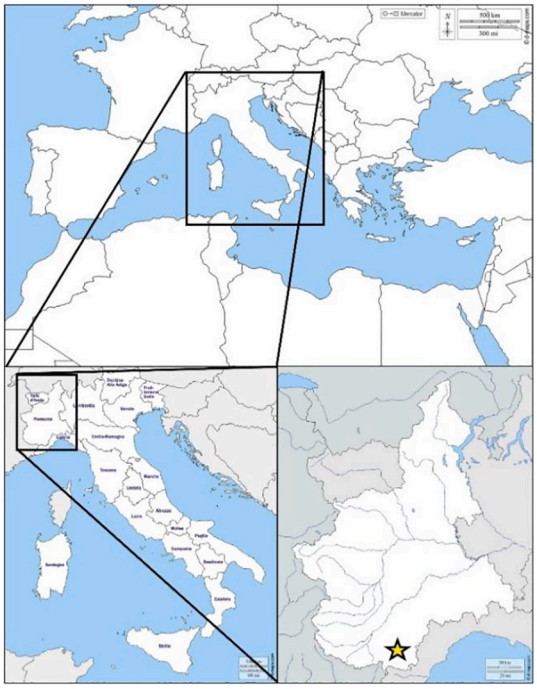


Fig. 1. Location of the sampling area. Bossea Cave is located in SW Piedmont (Italy). (maps used for the plate and modified, retrieved from: https://d-maps.com/carte.php?num_car=3126&lang=en, https://d-maps.com/carte.php?num_car=4828&lang=it and https://d-maps.com/carte.php?num_car=8256&lang=en).

Struttura Operativa Bossea CAI and by the Department of Environment, Land and Infrastructure Engineering of the Politecnico di Torino, working together with ARPA Piemonte. Sampling of microplastics requires different approaches, depending on the diverse environment, and the number of the zone sampled varies considerably among studies (e.g. Hidalgo-Ruz et al., 2012). Different studies on MPs in marine sediments used bulk samples when MPs cannot be easily identified visually, for example when they are too small for a naked eye identification or when their abundance is low (Hidalgo-Ruz et al., 2012). Five superficial sediment samples (upper 5 cm) were collected near the tourist paths, in different 1 × 1 m areas of the cave (Fig. 2). One sample was taken in a non-touristic cave zone, visited only by researchers and speleologists, used also to reach the Secondary Lab (Fig. 2). About 300 g of superficial sediment for each sampling area were collected with a metal spoon and placed in glass boxes. Although it is acknowledged that a low volume of sediment could be not representative of sediments at individual sampling stations, cave environment and concreting of cave sediments limited the volume of sediment that was available per site. All sampling material was pre-cleaned with ethanol and distilled water, and the samples were stored in the fridge at 6 °C until laboratory analysis.

2.2. Comparison between different methodologies for detecting MPs and laboratory tests

As MP studies in caves are limited, several methodologies to detect MPs from sediment sampling of different environments were taken as references (Table 1) and modified to obtain a cave-adapted method to detect MPs from cave sediments (see below). Some laboratory tests were also necessary to clarify some steps. The methodologies used for MP analysis are not standardised, but the protocols can be divided into different stages, as reported in Table 1.

To minimise MP samples contamination, non-plastic materials must be used from sampling to analysis. However, in extreme environments, the use of plastic containers for the collection of samples is also

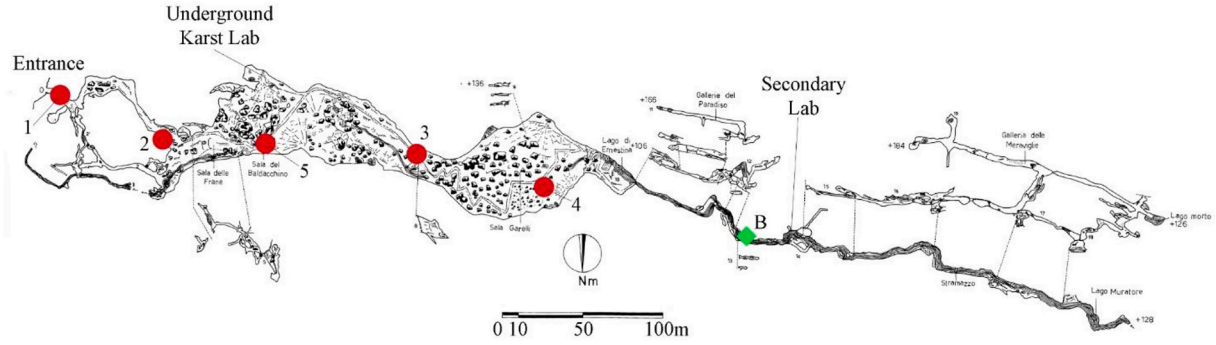


Fig. 2. Bossea Cave sampling areas. Red circles for microplastic sampling in the touristic zone, green turbot for microplastic sampling in the speleological tract to reach the Secondary Lab. Survey by Elia and Calleris (1988), modified. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Summary of the methodologies implemented in various studies that analysed MP contamination in sediments samples.

Location/ Origin	Sample type	Pre-treatment	Separation	Filtration	Post-treatment	MP observation, abundance and identification	References
China	Land sediments	30% KOH: NaClO; Ultrasonic bath	Centrifugation; density separation with NaCl solution and ZnCl2	0.45 μm membrane filtration	–	Stereomicroscope; Raman microscope	Zhou et al. (2019)
Canada	Intertidal marine sediment	30% H2O2	Density separation with NaCl solution	0.8 μm pore size, 47 mm gridded nitrocellulose filter	–	Motic Dissecting Microscope using 2× magnification	Mathalon and Hill (2014)
Indonesia	Marine sediment	H2O2	Density separation with concentrated NaCl solution	0.45 μm pore size, 47 mm cellulose filter paper	–	Nikon Eclipse E600 microscope	Cordova and Wahyudi (2016)
Italy	Lagoon sediment	–	Density separation with NaCl solution	0.7 μm pore size, 47 mm fiberglass filter	–	μFTIR spectroscopy; ESEM-EDS	Vianello et al. (2013)
China	Coastal marine sediment	–	Density separation with NaCl solution	Paper filter	–	Fluorescence microscope; μFTIR spectroscopy	Qiu et al. (2015)
China	Offshore sediment	30% H_2O_2	Density separation with NaI solution	8-μm pore size, 47 mm cellulose nitrate filter paper	–	Digital microscope equipped with MvImage software; μFTIR spectroscopy	Zhang et al. (2019)
Sigma-Aldrich (USA); Portugal; Store-bought or consumer; Portugal	Virgin polymers; Beach; Textile fibres; river water; river sediment	Dye: Acridine Orange, Basic Blue 24, Crystal Violet, Lactophenol Blue, Neutral Red, Nile Red, Safranin-T, Trypan Blue; For sediment only: wash with acid and distilled water	Density separation with NaCl solution	Filters of quartz (2.2 μm), glass microfibre (1.2 μm), nitrocellulose (0.2 μm), mixed cellulose esters (0.45 μm), black polycarbonate filters (PCTE, 0.2 μm), C18 octadecyl (12 μm)	15% H_2O_2 and 0.05 M iron catalyst (Fe)	Photographs and automated counting software (MP-VAT in Image-J); FTIR-ATR spectroscopy	Prata et al. (2019)

acceptable, taking into account the hypothetical contamination.

The majority of the considered papers used pre- or post-treatments to eliminate organic matter, applying 15% or 30% H_2O_2 solutions. No kind of pre-treatment was tested on our samples to minimise sample manipulation. However, organic matter removal with H_2O_2 solution was applied on filters during post-treatment tests.

The most used procedure in the considered papers to separate MPs from sediments is density separation with NaCl solution. Therefore, this stage was used to separate MPs from cave sediments. The specific density of MPs can vary considerably depending on the type of the polymer, the manufacturing process and the environmental factors as biofouling (Hidalgo-Ruz et al., 2012). Density values for concentrated saline NaCl solution is 1.20 g cm^{-3}, consequently denser polymers could be excluded (for specific densities of plastic polymers see Crawford and Quinn, 2016; Hidalgo-Ruz et al., 2012).

Different kind of filters, such as quartz, glass microfiber, nitrocellulose, cellulose, polycarbonate, and C18 octadecyl, as well as various porosities (from 0.2 to 12 μm), were used in the examined papers. A 0.7- and 1.2-μm pore glass microfiber filters (Whatman, 47 mm) were tested.

The organic matter on the dried filters was removed through the application of 0.5 ml of 15% and 30% hydrogen peroxide solution, left to react for 30 min and dried again for 1 h at 50 °C. The microplastics on dried filters were counted before and after the application of the hydrogen peroxide solutions to highlight the importance of organic matter removal.

Fluorescent whitening agents (FWAs) are often used in the production of plastic (Qiu et al., 2015), for example to brighten colours and mask the natural yellowing of plastics. Therefore, plastics with FWAs can absorb ultraviolet light (300–400 nm) and radiate purple (400–450 nm) or blue (450–480 nm) fluorescence (Qiu et al., 2015). Although MPs with FWAs can easily be detected under a fluorescence microscope, such equipment cannot be afforded by many laboratories. Therefore, a low-cost and easy to use ultraviolet (UV) flashlight can be used to irradiate the MPs (e.g. Ehlers et al., 2020; Klein and Fischer, 2019). However, a lot of organic and inorganic things could be fluorescent under a UV light, therefore, the use of this kind of flashlight has to be combined

with the microscopy for MPs detection. Different kind of flashlights can be used, depending on the size of the surface to observe and on the comfort of the working environment. Here, 365-nm (Alonefire SV10) and 395-nm (Youthink 100 LEDs) UV flashlights were tested on cave sediments and on filters in the laboratory.

2.3. Standardisation of the methodology for detecting MPs in cave sediments

The MP laboratory analysis consisted of two steps: extraction-purification and identification.

During sampling and laboratory analysis, all researchers used nitrile gloves and cotton coats, and all working surfaces were wiped with ethanol and distilled water to avoid MP contamination. The use of plastic equipment was avoided or replaced with non-plastic utensils. According to Cabrera et al. (2020), reducing the number of analytical steps when working with an environmental matrix where a low level of MP contamination is expected is fundamental. Therefore, we decided to not use sieves and abrasive substances, reducing the preparation time and decreasing the risk of MP contamination.

Samples were weighted before and after drying process. The sediments were placed in an aluminium box, covered with aluminium foil and dried into an oven at 40 °C to constant weight.

For each dried sample, three sub-samples of 10 g were selected via coning and quartering and poured in precleaned glass beakers. Sand and minerals were then removed by density separation. For each beaker, 100 ml of NaCl solution (200 g NaCl/0.6 l, density 1.22) was added, and the mixture was stirred with a magnetic mixer for 2 min. After settling for 24 h, the supernatant (30 ml) was then extracted with a glass pipet and filtered by a vacuum pump through a 1.2-μm pore size glass microfiber filter (Whatman, Ø 47 mm). Filters were placed on glass petri dishes covered with aluminium foil and dried in an oven at 40 °C for 2 h. Dried filters were subjected to organic matter removal through the application of 0.5 ml of 15% hydrogen peroxide solution, left to react for 30 min and dried again for 1 h at 50 °C.

The MPs on filters were observed alongside with and without a 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 2.5x zoom. The UV flashlight was positioned on a self-built pedestal with an inclination of 45°. Most filters were observed before and after organic matter removal to highlight the importance of the process in MP identification. On each filter, 56 rectangular areas were defined to count MPs (Supplementary Fig. 1). Visual identification was used to count MPs according to the following strict selection criteria to avoid misidentification of MPs: no cellular or organic structures must not be observable, fibres must have equally diameter throughout their entire length, particles must present clear and unvaryingly colours, and, if they are red fibres or transparent or white particles, they must be examined under high-magnification or a fluorescence microscope (Crawford and Quinn, 2016; Hidalgo-Ruz et al., 2012; Noren, 2007). The effectiveness of visual identification depending on the size of the examined item, consequently, the accuracy in visually identifying very small MPs is less reliable than with larger items (Hidalgo-Ruz et al., 2012; Song et al., 2015). Thus, a cut off of the particles smaller than 0.1 mm was made as suggested in European Commission (2013) and particles that could not be reliably identified as MPs were not take into consideration.

All counted MPs were described using the standardised size and colour sorting system (SCS) for categorising MPs (Crawford and Quinn, 2016); where necessary, higher magnifications were used to better understand fibre structures, with the help of x-sectional and longitudinal microscopic image of natural and synthetic fibres (Khan et al., 2017).

3. Results and discussion

3.1. Laboratory tests

Both 0.7- and 1.2-μm pore glass microfiber filters (Whatman, 47 mm) tested revealed the presence of MPs in the samples examined. However, 0.7-μm pore glass microfiber filters were less flattened and retained considerably more residue, making the visualisation and identification of MPs under the microscope more difficult. Therefore, 1.2-μm pore glass microfiber filters are recommended for 5- to 0.1-mm MPs identification in cave sediments.

Treatment of samples with chemicals has been used to remove organic materials, however, they may lead to a partial or complete degradation of microplastic too (Duis and Coors, 2016; Nuelle et al., 2014; Rocha-Santos and Duarte, 2015). When observing and counting the MPs on filters before and after the application of the hydrogen peroxide solutions, different problematics were detected. According to Nuelle et al. (2014), 30% hydrogen peroxide solution dissolves smaller MPs and damages the other ones, and MP fluorescence under the microscope was less intense in both cases. Using 15% H_2O_2 resulted in an acceptable compromise and produced remarkable results. Fluorescent fibre abundance before and after organic matter removal was performed on 11 filters to better understand the fluorescent organic and synthetic fibre percentages. The results are reported in the Supplementary Table. A percentage between 25 and 86.4% of fluorescent fibres was eliminated after organic matter removal.

The UV flashlight tests on filters revealed fundamental information. On the same filters and on the same areas of the cave, the 395-nm UV flashlight did not evidence the presence of any MPs, whereas the 365-nm UV light perfectly highlighted all fluorescent particles (Supplementary Fig. 2). Much attention needs to be paid to this aspect of the methodology, and it is advisable to do several tests before counting microplastics. However, the 365-nm UV light gave excellent results for MP counting on dried filters.

3.2. First evidence

Overall, 707 MPs were counted on 18 filters, 15 from the sediments of the touristic area and 3 from sediments collected in the speleological traits of the cave that contain the internal laboratory. Microplastics were found in all sediment samples, non-touristic traits included. However, the non-touristic sediment sample had about one third of the MP average value found in the samples of the tourist section of the cavity. The MP abundance, their shape and fluorescence are shown in Table 2 and Fig. 3.

Fibers (Fig. 4A, B, C, D) represented the majority of the MPs present in the cave sediments, respectively 14.4% between 1 and 5 mm and 70.6% between 0.1 and 0.99 mm of length, followed by fragments (14.3% microfragments and 0.3% fragments) (Fig. 4E and F). Some microbeads (0.1%) and microfilms (0.3%) were also observed, whereas no foam was found in cave sediments. As Bossea Cave is located in a relatively untouched valley, with only a small mountain village, we suggest that the MPs discovered in the cave are mainly originated from the daily tourist activities in the show cave. Globally, more than 60% of all textiles are produced from synthetic fibres (Henry and Klepp, 2018), and therefore, the shedding of fibres from clothes could be the sources of the MPs discovered in the cave. Fragments were also present in Bossea Cave sediments, especially in sampling area 4. Probably, MP fragments were produced during the electric system works, near the sampling area.

Fig. 3A shows the different sizes of the collected MPs. According to Crawford and Quinn (2016), MPs are categorised in two different classes: microplastics (1–5 mm) and mini-microplastics (1 mm–1 μm). In this investigation, mini-MPs accounted for 85.4% of the total MPs found in cave sediments, of which 58.4% were shorter than 0.5 mm.

The average MP abundance of each sampling area and their different types are shown in Table 3. The abundance of microplastics along the

Table 2
Abundance, shape and fluorescence of MPs in Bossea Cave sediments.

Filter	MP abundance	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
1.1	46	43	3	4	41	0	1	0	0	0	0	0	0
1.2	35	30	5	3	32	0	0	0	0	0	0	0	0
1.3	51	46	5	12	39	0	0	0	0	0	0	0	0
2.1	38	30	8	3	34	0	1	0	0	0	0	0	0
2.2	20	14	6	5	14	0	0	0	1	0	0	0	0
2.3	31	30	1	1	30	0	0	0	0	0	0	0	0
3.1	31	29	2	5	26	0	0	0	0	0	0	0	0
3.2	33	23	10	11	19	0	2	0	0	0	1	0	0
3.3	38	36	2	11	27	0	0	0	0	0	0	0	0
4.1	54	48	6	4	41	0	9	0	0	0	0	0	0
4.2	150	143	7	5	90	2	54	0	0	0	0	0	0
4.3	57	51	6	2	27	0	28	0	0	0	0	0	0
5.1	28	24	4	5	23	0	0	0	0	0	0	0	0
5.2	24	18	6	6	17	0	1	0	0	0	0	0	0
5.3	23	20	3	6	17	0	0	0	0	0	0	0	0
B1	13	11	2	6	4	0	2	0	1	0	0	0	0
B2	23	16	7	10	10	0	3	0	0	0	0	0	0
B3	12	8	4	3	8	0	1	0	0	0	0	0	0
TOT	708	620	87	102	499	2	102	0	2	0	1	0	0
%	100	87.7	12.3	14.4	70.5	0.3	14.4	0.0	0.3	0.0	0.1	0.0	0.0

tourist path varied from 2500 to 8700 items/kg. The lowest MP abundance in the cave sediments was found in sampling site 5. The number of MP particles was higher in sampling areas 1 and 4, the monitoring zones that were nearest and furthest to the cave entrance. These data could be related to the air circulation in the cave, which is stronger near the entrance because of air exchange with the outside and the narrowing of the vessel, and near the end of the touristic traits because of the presence of the waterfall and the narrowing of the cave. Otherwise, these data could be related to the time of tourist visits to the cave. Probably, these results are linked to all of these and other factors, which should be studied in more detail to better understand the MP pollution in caves. In the cave sediment sample taken from the non-touristic but speleological area, we found a mean of 1600 items/kg.

Comparisons with other cave sediment abundances are not possible at the moment, being this work the first on cave sediments. Moreover, a standardized method for assessing microplastics in sediments do not exist. However, despite different measurement methods and treatments greatly affect the results, some considerations about MPs abundance in sediments of different environments can be equally done. The MP abundance values found in the show cave are similar to those found in coastal marine sediments of China (5020 to 8720 items/kg) (Qiu et al., 2015) or in the intertidal zone of beaches along Nova Scotia (2000 to 8000 items/kg) (Mathalon and Hill, 2014). However, our MP values are high compared to those found in the Lagoon of Venice sediments (672–2175 items/kg) (Vianello et al., 2013), in Scapa Flow, Orkney (730–2300 items/kg) (Blumenröder et al., 2017), or in marine and freshwater sediments from different parts of the world (30–1900 items/kg) (e.g. Ballent et al., 2016; Matsuguma et al., 2017; Phuong et al., 2018; Tsang et al., 2017). This fact could be linked to the confined cave environment, which, in many areas, is hardly influenced by air currents or water flows, favouring MP deposition.

The highest MP abundance was fluorescent under UV light (87.7%) (Fig. 4C, D, E, F). Exploiting fluorescent whitening agents (FWAs) in plastics, using an inexpensive UV flashlight, facilitates the finding of MPs in cave sediments. Otherwise, according to Qiu et al. (2015), there are also several limitations because not all plastic contain FWAs, and different organic and inorganic matter compounds could be misunderstood as MPs. In fact, 12.3% of the total MPs in Bossea Cave sediments were not fluorescent (Fig. 4A and B), and twice the amount of the fibres would have been counted without organic matter removal. Transparent and white-coloured fibres are extremely difficult to separate from organic structures; however, excluding them, it is possible to underrepresent the MPs in the sample (Crawford and Quinn, 2016). Therefore, organic matter removal is a necessary step in MP determination in cave sediments. Visual identification method for MPs pollution is one of the most commonly used (e.g. Alomar et al., 2016; Cannas et al., 2017; Guerranti et al., 2017; Hidalgo-Ruz et al., 2012; Mathalon and Hill, 2014; Van Cauwenberghe et al., 2013) and it is an important, inexpensive method, especially for high volume samples (Crawford and Quinn, 2016). However, it is susceptible to operator biases and errors, thus frequently leading to misidentification (Crawford and Quinn, 2016; Prata et al., 2019), and it not allow to identify the polymers composition. A highly detailed examination with a high-magnification microscope or fluorescence microscopy can be performed, and staining techniques and spectroscopy can be used to confirm MP presence in the samples. Therefore, in different comparisons between microscopic and spectroscopic identification, overestimation or underestimation of MPs has been observed for both techniques (e.g. Hidalgo-Ruz et al., 2012; Song et al., 2015). The identification of individual particles by spectroscopic techniques is very time consuming and the spectra of samples are difficult to match with those of the library with high percentages, especially due to the contaminated surfaces of plastics (Song et al., 2015). Therefore, often only a small part of the sample is analysed (on average, from 1 to 10%), and the result is extrapolated to the complete sample (International Organization for Standardization and European Committee for Standardization, 2020). The ability to visually

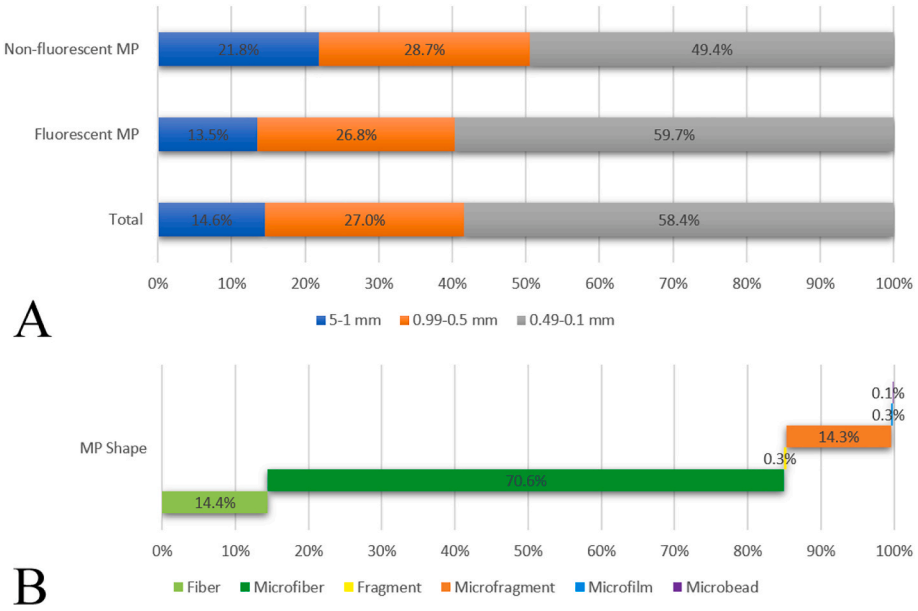


Fig. 3. Microplastic (MP) size and shape of Bossea Cave sediments. A: size percentages. B: shape percentages.

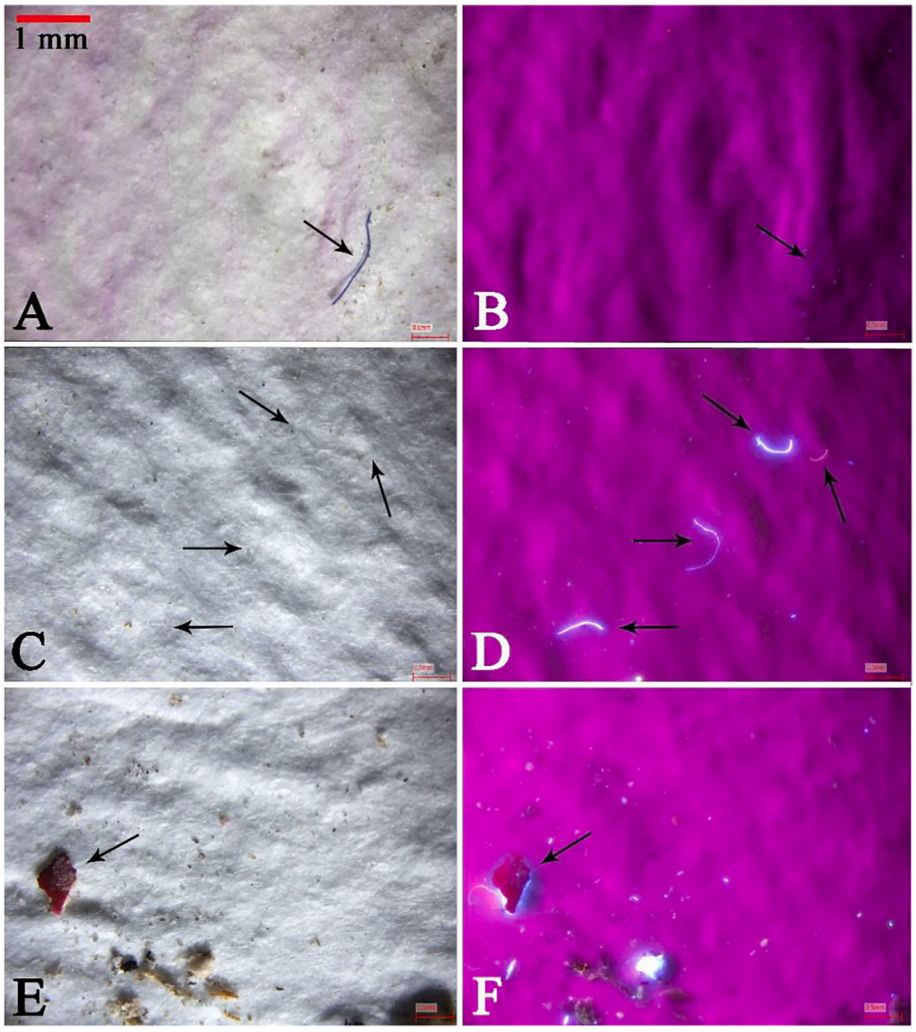


Fig. 4. Microplastic particles on filters observed with and without a UV flashlight under a microscope. A–B: blue microplastic fibre, non-fluorescent under UV flashlight. C–D: transparent microplastic fibres, fluorescent under UV flashlight. E–F: red microplastic fragment visible with and without UV flashlight. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
MP abundance in cave sediments and their shape [items/10 g of sediment].

Area	MP abundance average	Fibre FB	Microfibre MFB	Fragment FR	Microfragment MFR	Film FI	Microfilm MFI	Pellet PT	Microbeads MBD	Foam FM	Microfoam MFM
1	44.0	6.3	37.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
2	29.7	3.0	26.0	0.0	0.3	0.0	0.3	0.0	0.0	0.0	0.0
3	34.0	9.0	24.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0
4	87.0	3.3	52.7	0.7	30.3	0.0	0.0	0.0	0.0	0.0	0.0
5	25.0	5.7	19.0	0.1	3.9	0.0	0.0	0.0	0.0	0.0	0.0
Not touristic	16.0	6.3	7.3	0.0	2.0	0.0	0.3	0.0	0.0	0.0	0.0
Touristic path	43.9	5.5	31.8	0.1	7.1	0.0	0.1	0.0	0.0	0.0	0.0

distinguish MPs from natural particles becomes difficult for smaller pieces, and therefore, a cut-off of particles less than 0.1 mm was applied, as suggested by the European Commission (2013). Probably, a set of several methodologies is the optimal choice. Being the characteristics of microplastics different among samples, an initial screening analysis using spectroscopy methods could be conducted, to create a criteria for the identification of major microplastics, followed by microscopic analysis to determine the microplastic abundance, as suggested in Song et al. (2015). However, thanks to the use of the organic matter removal, the UV flashlight, and the cut-off the particles less than 0.1 mm, the possible overestimation of the microplastics has been greatly reduced.

Fluorescent MPs can be photographed and automatically quantified by a counting software, limiting subjectivity in the quantification (Prata et al., 2019). Different counting software packages will be tested in the future to better represent the percentages of MPs in show caves.

Figs. 5 and 6 shows the different colours of the collected microplastic fibres. Of the fluorescent fibres, 84% (Fig. 5) were transparent, followed by beige (4.6%), blue (3.6%), pink (1.9%) and red (1.5%) ones. Fibres with other colours accounted for less than 1% of the total fluorescent fibres. Non-fluorescent MP fibres (Fig. 6) were mainly blue (46.1%) or black (22.4%), followed by grey, brown and red ones (between 5 and 7%). The colour of microplastics often provides an indication of the chemical pollutants which contaminated them. Different researchers found high levels of pollutants on yellow and black microplastics (e.g. Frias et al., 2010; Karapanagioti et al., 2011). In Bossea cave, about a fifth of non-fluorescent fibres found is black, therefore, further investigations should be carried out in the future. Moreover, the colour of microplastics can be correlated with the consumption by organisms (e.g. Carpenter et al., 1972; Romeo et al., 2015), being confused with natural food. In natural caves there is no light, therefore, the colour of MP fibres could be not of particular interest for cave animal ingestion. However, they might be attracted by fluorescent fibres. Moreover, the colour of MPs found in Bossea cave could be equally interesting for the external fauna, especially aquatic organisms, which could be subjected to contamination by microplastics transported from the inner stream of the cave.

The deterioration of the fibres depends on a combination of factors as biodeterioration, temperature, pH and relative humidity of the environment, moisture, reactivity and nature of the material and its progressive natural ageing (Carr, 2017; Szostak-Kotowa, 2004). Biological attack is caused by bacteria and fungi activities, insects and vertebrates (Carr, 2017). The synthetic fibres are generally considered to be resistant to bacteria, mould and mildew activities, however certain treatments used on textile products, staining with organic material or blending with a more susceptible fibre can promote growth (Szostak-Kotowa, 2004). Moreover, mould and mildew grow increases in higher relative humidity environments (Szostak-Kotowa, 2004). Microbial growth causes loss of strength, elongation and discolouration (Szostak-Kotowa, 2004). Therefore, the high number of transparent fibres found in Bossea cave could be related to biodeterioration, given also the high relative humidity of the cave environment.

Bossea Cave is rich in troglobitic and endemic fauna, and MPs can be ingested by cave animals and endanger the fragile ecosystems of the caves. Geologic features are the primary attraction of the show cave, and MPs can irreversibly damage speleothems deposited on them and on the paleontological remains. Microplastics can directly damage speleothems, being incorporated into the cave formation growth, sometimes colouring them, or indirectly, by providing nutrients for acid-producing organisms that can dissolve limestone (Jablonsky et al., 1993). Moreover, the cavity is crossed by a subterranean river that directly flows in

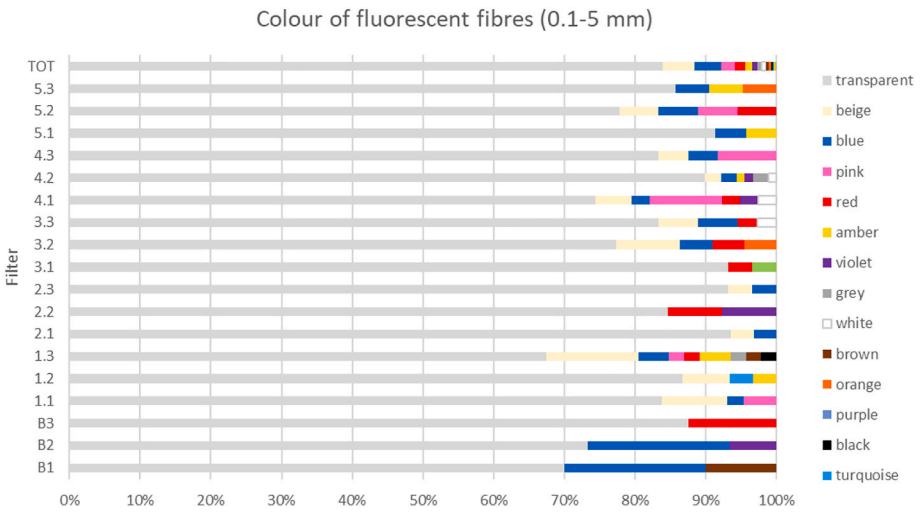


Fig. 5. Percentages of the colours of fluorescent synthetic fibres on filter from sediments sampled in different Bossea Cave areas. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

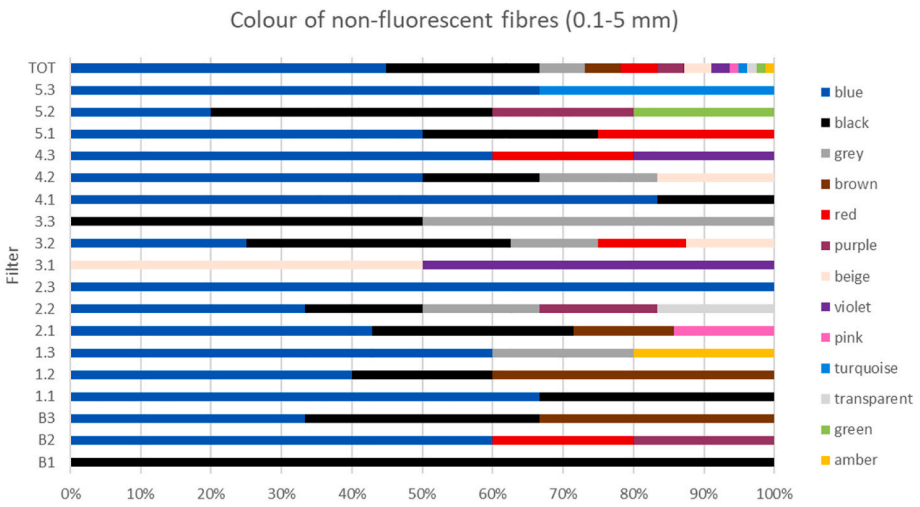


Fig. 6. Percentages of colours of non-fluorescent synthetic fibres on filter from sediments sampled in different Bossea Cave areas. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the Corsaglia River, and MPs can pollute karst aquifers. It must be considered that karst aquifers are open systems, even susceptible to contamination by surface pollutants, and therefore, the areas above the caves must also be monitored.

4. Conclusion

This study documented the presence of MPs in the examined show cave, filling a gap in the study of microplastic pollution, providing useful references for further research. A valid non-invasive and minimally manipulative technique to separate and detect MPs in cave sediments was tested. This method is eco-friendly, incurs low costs, and the equipment is easily obtainable. From the analysis on cave sediments, fibre-shaped and mini-microplastics dominated the samples, suggesting that synthetic clothes of visitors are the main source of microplastic pollution in cave. Microplastics in cave could contaminate potable water, polluting watercourses and nearby environments and irremediably damage speleothems and cave ecosystems. The methodologies that enable the detection of MP contamination are crucial to understand the gravity of the problem and define strategies for cave conservation. Substantial efforts must be made to protect caves resources, implementing new strategies and providing education. Cave conservation should become a priority for the management of the cave resources.

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.

Acknowledgments

The authors are grateful to the Bossea Cave managers, especially to Claudio Camaglio, Paola Marini for useful comments and ideas on the manuscript, Giovanna Zanetti for chemical advice, Claudio De Regibus for helping with laboratory equipment, Stefano Vanin for providing us useful forensic science materials and Jo De Waele, Marco Isaia and Vento Cave managers for 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].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.118261.

Credit author statement

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

References

Alomar, C., Estarellas, F., Deudero, S., 2016. Microplastics in the Mediterranean Sea: deposition in coastal shallow sediments, spatial variation and preferential grain size. Mar. Environ. Res. 115, 1–10. https://doi.org/10.1016/j.marenvres.2016.01.005.

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.

Antonellini, M., Nannoni, A., Vigna, B., 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. https://doi.org/10.1016/j.geomorph.2019.07.019.

Baker, G.M., Roberts, B., 2015. Making Cave Lint Camps a Success, 21st National Cave and Karst Management Symposium, Cave City. Kentucky, USA, pp. 60–62.

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.

Blumenröder, J., Sechet, P., Kakkonen, J.E., Hartl, M.G., 2017. Microplastic contamination of intertidal sediments of Scapa Flow, Orkney: a first assessment. Mar. Pollut. Bull. 124, 112–120. https://doi.org/10.1016/j.marpolbul.2017.07.009.

Cabrera, M., Valencia, B.G., Lucas-Solis, O., Calero, J.L., Maisincho, L., Conicelli, B., Moulatlet, G.M., Capparelli, M.V., 2020. A new method for microplastic sampling and isolation in mountain glaciers: a case study of one antisana glacier, Ecuadorian Andes. Case Stud. Chem. Environ. Eng. 2, 100051. https://doi.org/10.1016/j.cscee.2020.100051.

Cannas, S., Fastelli, P., Guerranti, C., Renzi, M., 2017. Plastic litter in sediments from the coasts of south Tuscany (Tyrrhenian Sea). Mar. Pollut. Bull. 119, 372–375. https://doi.org/10.1016/j.marpolbul.2017.04.008.

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.

Carr, D., 2017. Forensic Textile Science. Woodhead Publishing.

Chelius, M.K., Beresford, G., Horton, H., Quirk, M., Selby, G., Simpson, R.T., Horrocks, R., Moore, J.C., 2009. Impacts of alterations of organic inputs on the bacterial community within the sediments of Wind Cave, South Dakota, USA. Int. J. Speleol. 38, 1–10.

Christman, A., 2019. Cave Dwelling Dust Bunnies: Lint Accumulation and Microplastics in Lewis and Clark Caverns State Park (PhD Thesis).
Cigna, A.A., 2016. Tourism and show caves. Z. Geomorphol. 60, 217–233. https://doi.org/10.1127/zfg_suppl/2016/00305.
Cigna, A.A., Forti, P., 2013. Caves: the most important geotouristic feature in the world. Tourism and Karst areas 6, 9–26.
Cincinelli, A., Scopetani, C., Chelazzi, D., Lombardini, E., Martellini, T., Katsoyiannis, A., Fossi, M.C., Corsolini, S., 2017. Microplastic in the surface waters of the Ross Sea (Antarctica): occurrence, distribution and characterization by FTIR. Chemosphere 175, 391–400. https://doi.org/10.1016/j.chemosphere.2017.02.024.
Corami, F., Rosso, B., Bravo, B., Gambaro, A., Barbante, C., 2020. A novel method for purification, quantitative analysis and characterization of microplastic fibers using Micro-FTIR. Chemosphere 238, 124564. https://doi.org/10.1016/j.chemosphere.2019.124564.
Cordova, M.R., Wahyudi, A., 2016. Microplastic in the deep-sea sediment of Southwestern Sumatran waters. Mar. Res. Indones. 41, 27–35. https://doi.org/10.14203/mri.v41i1.99.
Crawford, C.B., Quinn, B., 2016. Microplastic Pollutants. Elsevier Limited.
Duis, K., Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ. Sci. Eur. 28, 1–25. https://doi.org/10.1186/s12302-015-0069-y.
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.
Elia, E., Calleris, V., 1988. Grotta di Bossea, Mondo ipogeo. Cuneo 5–10.
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. In: Ispra: European Commission, vol. 126. Joint Research Centre. https://doi.org/10.2788/99475. MSFD Technical Subgroup on Marine Litter.
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.
Guerranti, C., Cannas, S., Scopetani, C., Fastelli, P., Cincinelli, A., Renzi, M., 2017. Plastic litter in aquatic environments of Maremma regional park (Tyrrhenian sea, Italy): contribution by the Ombrone river and levels in marine sediments. Mar. Pollut. Bull. 117, 366–370. https://doi.org/10.1016/j.marpolbul.2017.02.021.
Henry, B., Klepp, I.G., 2018. Microplastic Pollution from Textiles: A Literature Review. Project Report no.1–2018. Consumption Research Norway-SIFO, Oslo.
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.
International Organization for Standardization, European Committee for Standardization, 2020. Plastics - Environmental Aspects - State of Knowledge and Methodologies. CEN ISO/TR 21960:2020). 2020, B.S.L.
Jablonsky, P., Kraemer, S., Yett, B., 1993. Lint in Caves. In: National Cave Management Symposium, pp. 73–81. Carlsbad, NM.
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.
Jiang, C., Yin, L., Li, Z., Wen, X., Luo, X., Hu, S., Yang, H., Long, Y., Deng, B., Huang, L., 2019. Microplastic pollution in the rivers of the Tibet plateau. Environ. Pollut. 249, 91–98. https://doi.org/10.1016/j.envpol.2019.03.022.
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.
Khan, A., Abir, N., Rakib, M.A.N., Bhuiyan, E.S., Howlader, M.R., 2017. A review paper on textile fiber identification. IOSR Journal of Polymer and Textile Engineering (IOSR-JPTE) 4, 14–20. https://doi.org/10.9790/019X-04021420.
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.
Mathalon, A., Hill, P., 2014. Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. Mar. Pollut. Bull. 81, 69–79. https://doi.org/10.1016/j.marpolbul.2014.02.018.
Matsuguma, Y., Takada, H., Kumata, H., Kanke, H., Sakurai, S., Suzuki, T., Itho, M., Okazaki, Y., R, B., Zakaria, M.P., Weerts, S., Newman, B., 2017. Microplastics in sediment cores from Asia and Africa as indicators of temporal trends in plastic pollution. Arch. Environ. Contam. Toxicol. 73, 230–239. https://doi.org/10.1007/s00244-017-0414-9.
Noren, F., 2007. Small Plastic Particles in Coastal Swedish Waters. N-Research Report, commissioned by KIMO, Sweden.
Nuelle, M.-T., Dekiff, J.H., Remy, D., Fries, E., 2014. A new analytical approach for monitoring microplastics in marine sediments. Environ. Pollut. 184, 161–169. https://doi.org/10.1016/j.envpol.2013.07.027.
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.
Pate, D., 1999. Successful Cave Management Strategies at Carlsbad Caverns National Park. In: 14th National Cave and Karst Management Symposium, pp. 148–151. Chattanooga.
Phuong, N.N., Poirier, L., Lagarde, F., Kamari, A., Zalouk-Vergnoux, A., 2018. Microplastic abundance and characteristics in French Atlantic coastal sediments using a new extraction method. Environ. Pollut. 243, 228–237. https://doi.org/10.1016/j.envpol.2018.08.032.
Prata, J.C., Reis, V., Matos, J.T., da Costa, J.P., Duarte, A.C., Rocha-Santos, T., 2019. A new approach for routine quantification of microplastics using Nile Red and automated software (MP-VAT). Sci. Total Environ. 690, 1277–1283. https://doi.org/10.1016/j.scitotenv.2019.07.060.
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.
Rocha-Santos, T., Duarte, A.C., 2015. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. Trac. Trends Anal. Chem. 65, 47–53. https://doi.org/10.1016/j.trac.2014.10.011.
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.
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.
Szostak-Kotowa, J., 2004. Biodeterioration of textiles. Int. Biodeterior. Biodegrad. 53, 165–170. https://doi.org/10.1016/S0964-8305(03)00090-8.
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.
Van Cauwenberghe, L., Vanreusel, A., Mees, J., Janssen, C.R., 2013. Microplastic pollution in deep-sea sediments. Environ. Pollut. 182, 495–499. https://doi.org/10.1016/j.envpol.2013.08.013.
Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A., Da Ros, L., 2013. Microplastic particles in sediments of Lagoon of Venice, Italy: first observations on occurrence, spatial patterns and identification. Estuar. Coast Shelf Sci. 130, 54–61. https://doi.org/10.1016/j.ecss.2013.03.022.
Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031.
Zhang, C., Zhou, H., Cui, Y., Wang, C., Li, Y., Zhang, D., 2019. Microplastics in offshore sediment in the yellow Sea and east China Sea, China. Environ. Pollut. 244, 827–833. https://doi.org/10.1016/j.envpol.2018.10.102.
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., 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.