The invisible environmental impact of tourism in show caves: microplastic pollution in three Italian show caves

area, a minimum of 150 g of superficial sediments (upper 5 cm) were collected. Bossea cave samples were collected once in 2021, instead Borgio Verezzi and Toirano cave samples were collected once in 2022. Being caves conservative environments (Chiarini et al., 2022), variations in environmental parameters are often minimal, unaffecting data on MPs, with exceptions for active caves. Moreover, sampling areas along the tourist paths are away from watercourses, which could seasonally modify the amounts of MPs in sediments. Sediment samples of Bossea cave were previously analyzed using the method described in Balestra & Bellopede (2022); samples collected in Borgio Verezzi and Toirano caves were analyzed according to this method, improved.

Separated particles on filters were observed 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, 4x, 10x or higher magnifications (Balestra & Bellopede, 2022). Detected MPs were characterized according to the Standardized size and colour sorting system (SCS) (Crawford & Quinn, 2016).

Fluorescent MPs on filters of Borgio Verezzi and Toirano caves were also counted and characterized by shape and size using the automated software MUPL (Giardino et al., 2023) through the creation of high-definition photographs under UV light. The parameters were set to have a total mean error <10% and an error on each filter <15%.

Finally, a portion of MPs found in the Toirano and Borgio Verezzi caves was verified using a micro-Fourier Transform Infrared Spectroscope (micro-FTIR) Shimadzu AIM-9000 microscope equipped with a Shimadzu IRTracer-100 spectrophotometer and a Shimadzu ATR with a germanium prism. Spectra were compared with the ATR-Polymer library, followed by a visual analysis comparison of characteristic bands in the reference spectrum, and accepted only with a match degree ≥ 80% (Fossi et al., 2017).

3. Results and discussions

MPs were found in all cave sediment samples, non-touristic areas included, with higher amount along the tourist paths (Table 1). MP pollution in the touristic areas of the caves could be related to the visitors presence, however, it is not possible to exclude a MP contamination from the external environments, due to the open nature of the karst systems (Balestra et al., 2023), especially in the cave of Borgio Verezzi, located in the town of Borgio, in which it is possible that some of the pollution comes from the roads above. In the Toirano caves, MP amount in the speleological area was similar to the touristic ones; this fact could be related to presence of beautiful speleothems in this easy-to-access area, often visited by speleologists and researchers which can carry particles in this zone. Moreover, washing of the tourist path with pumped water is carried out, which can move the deposited material, accumulate it in certain areas and/or remove it from others.

Table 1. MP abundances in sediments of the examined show caves

Show cave	Touristic area [items/kg]	Not-touristic area [items/kg]
Toirano caves	1060.0	1033.3
Borgio Verezzi cave	1103.3	666.7
Bossea cave	1906.7	733.3

Fibre was the most common shape among the MPs present in the sediments of all caves, followed by fragments and other shapes (Table 2). Up to 60% of world textiles production are synthetic (Boucher & Friot, 2017) suggesting that synthetic clothes of tourists could be the main source of pollution in show cave sediments.

Table 2. MP shape in the examined show caves

Show cave	Fibres	Fragments	Other
Toirano caves	93.7%	6.3%	0.0%
Borgio Verezzi cave	87.9%	12.1%	0.0%
Bossea cave	94.2%	5.5%	0.3%

MPs less than 1 mm accounted for more than 70% in sediments of all caves (Table 3).

Table 3. MP size in the examined show caves

Show cave	5-1 mm	0.99-0.4 mm
Toirano caves	25.3%	74.7%
Borgio Verezzi cave	27.5%	72.5%
Bossea cave	27.3%	72.7%

The fluorescent MP percentages varied for each cave with a mean value of about 74%, highlighting the importance of visual identification under microscope, in order to not lose the non-fluorescent particles (about 25%) during MP detection.

Spectroscopic analysis highlights the presence of polyamide, polyester, polyethylene, polyethylene terephthalate, polyvinyl acetate, polyacrylamide, ethylene vinyl alcohol, copolymer and polyvinyl formal in Borgio Verezzi cave sediments and polyester, polyethylene terephthalate, polyacrylamide, ethylene vinyl acetate, and polypropylene in Toirano caves ones. The MP identification by spectroscopy is useful to understand the possible source of pollution: many of the plastics found in Borgio Verezzi and Toirano caves are used in textile manufacturing, supporting the assumptions on the primary origin of MPs in cave sediments due to the tourist clothes.

4. Conclusion

This study highlights the presence of microplastics in deposits of all examined Italian show caves. To obtain as much information as possible, visual identification under microscope and MUPL automated software were used, showing the importance of combine different methodologies. Microplastics were present in each sampling area of all caves, with higher amount along the tourist paths. Most of the particles were fluorescent under UV light. Small size, fibre-shape, polyester and polyolefin microplastics dominate the samples, suggesting that synthetic clothes could be the main source of microplastic pollution in show caves. Microplastic monitoring in subterranean environments is a fundamental step to establish the current degree of pollution and consequently define strategies for the protection and management of this geological heritages.

5. Acknowledgments

The authors are grateful to the Toirano, Borgio Verezzi and Bossea caves managers. 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].

References

Ambrosini R., Azzoni R.S., Pittino F., Diolaiuti G., Franzetti A. and Parolini M. (2019), First evidence of microplastic contamination in the supraglacial debris of an alpine glacier, *Environmental pollution*, **253**, 297-301.

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

Balestra V., Bellopede R., Cina A., De Regibus C., Manzino A., Marini P., et al. (2021), Study of the environmental impact in show caves: a multidisciplinary research, *Geoingegneria Ambientale e Mineraria*, **163-164**, 24-35.

Balestra V., Vigna B., De Costanzo S. and Bellopede R. (2023), Preliminary investigations of microplastic pollution in karst systems, from surface watercourses to cave waters, *Journal of Contaminant Hydrology*, **252**, 104117.

Bertoldi C., Lara L.Z., Fernanda A.d.L., Martins F.C., Battisti M.A., Hinrichs R., et al. (2021), First evidence of microplastic contamination in the freshwater of Lake Guaíba, Porto Alegre, Brazil, *Science of The Total Environment*, **759**, 143503.

Boucher J. and Friot D. Primary microplastics in the oceans: a global evaluation of sources.^(2017), In. IUCN, pp. 43.

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

Calaforra J., Fernández-Cortés A., Sánchez-Martos F., Gisbert J. and Pulido-Bosch A. (2003), Environmental control for determining human impact and permanent visitor capacity in a potential show cave before tourist use, *Environmental Conservation*, **30**, 160-167.

Chelius M.K., Beresford G., Horton H., Quirk M., Selby G., Simpson R.T., et al. (2009), Impacts of alterations of organic inputs on the bacterial community within the sediments of Wind Cave, South Dakota, USA, *International Journal of Speleology*, **38**, 1-10.

Chiarini V., Duckeck J. and De Waele J. (2022), A Global Perspective on Sustainable Show Cave Tourism, *Geoheritage*, **14**, 1-27.

Christman A. Cave Dwelling Dust Bunnies: Lint Accumulation and Microplastics in Lewis and Clark Caverns State Park.^(2019), In, **Environmental Policy and Project Management** Carroll College, Helena, Montana.

Cigna A. (2013), Le grotte turistiche e la protezione dell'ambiente, EUT Edizioni Università di Trieste, 2013.

Cigna A.A. (2016), Tourism and show caves, *Zeitschrift für Geomorphologie*, **60**, 217-233.

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

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

Culver D.C. and Pipan T. (2019), The biology of caves and other subterranean habitats, Oxford University Press, USA.

Fossi M.C., Romeo T., Baini M., Panti C., Marsili L., Campani T., et al. (2017), Plastic debris occurrence, convergence areas and fin whales feeding ground in the Mediterranean marine protected area Pelagos sanctuary: a modeling approach, *Frontiers in marine science*, 167.

Giardino M., Balestra V., Janner D. and Bellopede R. (2023), Automated method for routine microplastic detection and quantification, *Science of The Total Environment*, **859**, 160036.

Gillieson D.S. (2011), Management of caves, Karst management, Springer, 2011, pp. 141-158.

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

Lang M., Faimon J. and Ek C. (2015a), A case study of anthropogenic impact on the CO2 levels in low-volume profile of the Balcarka Cave (Moravian Karst, Czech Republic), *Acta Carsologica*, **44**, 71-80.

Lang M., Faimon J. and Ek C. (2015b), The relationship between carbon dioxide concentration and visitor numbers in the homothermic zone of the Balcarka Cave (Moravian Karst) during a period of limited ventilation, *International Journal of Speleology*, **44**, 7.

Liu D., Zheng Y., Chen L. and Wen D. (2022), Prevalence of small-sized microplastics in coastal sediments detected by multipoint confocal micro-Raman spectrum scanning, *Science of The Total Environment*, **831**, 154741.

Mammola S. (2019), Finding answers in the dark: caves as models in ecology fifty years after Poulson and White, *Ecography*, **42**, 1331-1351.

Moldovan O.T., Bercea S., Năstase-Bucur R., Constantin S., Kenesz M., Mirea I.C., et al. (2020), Management of water bodies in show caves–a microbial approach, *Tourism Management*, **78**, 104037.

Neelavannan K., Sen I.S., Lone A.M. and Gopinath K. (2022), Microplastics in the high-altitude Himalayas: Assessment of microplastic contamination in freshwater lake sediments, Northwest Himalaya (India), *Chemosphere*, **290**, 133354.

Panno S.V., Kelly W.R., Scott J., Zheng W., McNeish R.E., Holm N., et al. (2019), Microplastic contamination in karst groundwater systems, *Groundwater*, **57**, 189-196.

Phuong N.N., Poirier L., Lagarde F., Kamari A. and Zalouk-Vergnoux A. (2018), Microplastic abundance and characteristics in French Atlantic coastal sediments using a new extraction method, *Environmental Pollution*, **243**, 228-237.

Piano E., Nicolosi G., Mammola S., Balestra V., Baroni B., Bellopede R., et al. (2022), A literature-based database of the natural heritage, the ecological status and tourism-related impacts in show caves worldwide, *Nature Conservation*, **50**, 159-174.

Romano E., Bergamin L., Di Bella L., Baini M., Berto D., D'Ambrosi A., et al. (2023), First record of microplastic in the environmental matrices of a Mediterranean marine cave (Bue Marino, Sardinia, Italy), *Marine Pollution Bulletin*, **186**, 114452.

Valentić L., Kozel P. and Pipan T. (2022), Microplastic pollution in vulnerable karst environments: case study from the Slovenian classical karst region, *Acta Carsologica*, **51**, 79-92.

Zhang Y., Gao T., Kang S., Allen S., Luo X. and Allen D. (2021), Microplastics in glaciers of the Tibetan Plateau: Evidence for the long-range transport of microplastics, *Science of The Total Environment*, **758**, 143634.