Summary

Microplastic (MP) pollution is a worldwide concern. MP were found in both terrestrial and aquatic environments, in atmosphere and in different species, raising significant environmental and public health concern. While this kind of pollution has been extensively studied in marine environments, terrestrial ones are less studied, and some habitats are just at an early stage. MP pollution in karst systems is still poorly studied, despite the presence of protected species and habitats, and important water reserves. Indeed, karst aquifers provide 25% of the global potable water, and are particularly vulnerable due to their intrinsic hydrogeological characteristics, which facilitate the direct infiltration and transport of contaminants, including MPs, from surface to groundwater. MPs can adsorb and be vectors for other pollutants, and are rich of additives, which are released into the environment and may be hazardous to habitats and species. Moreover, MPs can be directly or indirectly assimilated by organisms, leading to bioaccumulation and biomagnification. Therefore, understanding the extent of MP pollution in karst environments and associated issues is crucial for assessing risks, developing monitoring frameworks, and implementing effective management and mitigation strategies.

Recently, microfibres (MFs) have emerged as a new and concerning class of micropollutants too, threatening natural environments. Natural, regenerated, and synthetic MFs have been detected across various environmental compartments as well as within organisms. Although synthetic MFs are typically identified in MP analyses, natural and regenerated MFs are often overlooked or incorrectly classified as MPs. Despite being generally considered biodegradable, their degradation pathways and rates within ecosystems remain poorly understood, and their potentially faster degradation could lead to the release of toxic compounds, while their physical properties may contribute to long-term accumulation in the environment. Therefore, comprehending their potential hazards and the impact they may exert on ecosystems is crucial for effective environmental conservation and management.

This research aims to investigate MP and MF pollution in aquifers and karst systems, analysing waters, sediments and fauna, to understand the extent of the problem with a multidisciplinary point of view, providing new insights into their environmental impacts, and propose correct methods of monitoring and analysis. Monitoring was performed in different Italian Regions.

The following points were specifically addressed:

- Identification of suitable methods for monitoring MPs in karst systems, from sampling to laboratory analysis, microscopy and spectroscopy. In particular, methods for water and sediment analysis were defined.
- Investigations of MP and MF pollution in show caves
    - Investigations of MP and MF pollution in the karst system of Bossea cave, Piedmont, were done with a multidisciplinary approach. Sediments, water and fauna of the system were analysed to understand the extend of the problem. After the sediment analysis, the water of the system was analysed

from surface watercourses to springs, developing an extensive sampling inside the Bossea cave, in the speleological and tourist areas. A specimen of *Proasellus franciscoloi*, a stygobiont (i.e. specialist of underground water environments) crustacean, was analysed together with CNR-IRET and Università degli Studi di Firenze. Finally, the presence of bisphenols was investigated in the water of the system, to verify if there could be a correlation with the presence of microplastics. Analysis on these samples were conducted during my period abroad at Universitat de València.

  - The sediments of other two Italian show caves (Toirano and Borgio Verezzi, Liguria) were analysed, making comparison with Bossea cave. A software of image analysis was tasted for MP counting.
  - Dripping waters in different points of the Torri di Slivia cave, Friuli-Venezia-Giulia, were analysed to verify the presence of MPs and other microparticles of anthropogenic origin, suggesting possible source of pollution. This research was conducted together with Università degli Studi di Trieste and CNR-STIIMA.

- Investigations of MP and MF pollution in speleological caves and springs. Karst aquatic environments with protected at EU level stygobionts were investigated in the Italian sector of the Classical Karst, Friuli-Venezia-Giulia. Waters and submerged sediments of different speleological caves and springs were analysed to understand the extend of the problem. This research was conducted together with Università degli Studi di Milano and Charles University of Prague.
- Investigations of MP and MF pollution in unexplored caves. These environments are the last frontier in terms of human exploration. Therefore, several unexplored caves were sampled before the passage of speleologists to observe if microplastics and microfibers were already present, and, if so, assume the source of contamination and the probable transport matrix.
- Investigations of MP and MF pollution in different deep aquifers and springs of the Cuneo province, Piedmont, to check for contamination and identify possible sources. These analyses were carried out as a sort of white, as the waters of certain aquifers are extremely deep, checking the validity of the used methodology