## Preliminary research of microplastic from cave sediments, Croatia

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Introduction: Microplastics, plastic debris with a particle diameter of less than 5 mm, have attracted growing attention in recent years. Based upon size alone, microplastics are of special concern because they can be readily ingested, moving throughout the food web more easily than larger particles. Plastic pollution in both the marine and freshwater environments has intensified in the last decades due to accumulation of micro and nano particles, in water, organisms and food web. Based on the recent scientific data, microplastics have been detected throughout the marine food web from zooplankton to shellfish, fish and mammals. Microplastics have been found across all habitats in the ocean, including coral reefs, shallow bays, estuaries, the open ocean, and the deep sea.

The underground environment is still unexplored in terms of microplastic content. Caves are a common phenomenon in the Dinaric karst and in the Croatia, where there are more than 15,000 known caves and pits. Caves in Croatia are mostly freshwater, but there are also numerous anchialine caves located near the Adriatic coast where marine influence are notable also. Anchialine caves are defined as a tidallyinfluenced subterranean estuary located within crevicular and cavernous karst and volcanic terrains that extends inland to the limit of seawater penetration [1]. In some Croatian anchialine caves, elevated concentration of toxic metals have been observed [2,3]. However, plastic pollution in anchialine and freshwater caves is currently undocumented.

Methods: Sediment samples from 7 anchialine and freshwater caves were collected manually by experienced speleologists (Fig. 1). After drying, an initial disaggregation was done of the dried sediments. The disaggregated sediments were sieved using stacked 5-mm and 0.3-mm sieves Microplastics were collected on the 0.3-mm sieve and subjected to wet peroxide oxidation (WPO) in the presence of a Fe(II) catalyst to digest the labile organic matter. The plastic debris remained unaltered. The WPO mixture was subjected to density separation in NaCl(aq) to isolate the plastic debris through flotation. The floating solids were then separated from the denser undigested mineral components using a density separator. The floating plastic debris was collected in the density separator using a custom 0.3-mm filter and air-dried. Finally, the plastic material was removed and weighed to determine the microplastics concentration.



Fig. 1: Cave sediment sampling.

**Results and Discussion:** We have preliminary results of microplastic content in sediments from 7 freshwater and anchialine caves. Although, this is currently not enough data to determine the impacts of microplastics in the Croatian karst environment, it is sufficient to say that plastic has invaded this virgin environment.

## Acknowledgements:

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**References:** [1] Bishop et al. (2015) *J crustacean biology* 35:511-514; [2] Cuculić et al. (2011) *Estuarine, Coastal and Shelf Science* 151:10-20; [3] Kwokal et al. (2014) *Estuarine, Coastal and Shelf Science* 95:253-263;