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MICROPLASTIC POLLUTION IN VULNERABLE KARST ENVIRONMENTS: CASE STUDY FROM THE SLOVENIAN CLASSICAL KARST REGION

ONESNAŽENJE Z MIKROPLASTIKO V RANLJIVEM KRAŠKEM OKOLJU: PRIMER SLOVENSKEGA KLASIČNEGA KRASA

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Abstract UDC 551.435.8:504.5:691.175(497.47)
Lara Valentić, Peter Kozel, Tanja Pipan: Microplastic pollution in vulnerable karst environments: case study from the Slovenian classical karst region
Since the start of mass production of plastic materials more than a century ago, the problem of accumulating plastic waste in the environment has reached epic proportions. Recently, the problem of smaller plastic particles (microplastic, MP) in the environment has become a widely studied topic, but the amount and types of MP in karst environments are still poorly known. Thus, the objective of this study was to collect and analyse samples from various karst habitats and to try and determine the scope of pollution in karst springs that are in part used as sources for drinking water. Of the potential pollution sources, we sampled rainwater, two discharges from wastewater treatment plants, and a leachate from a landfill. We conducted polymer analyses of potential MP particles using FTIR-ATR. The results showed that eight samples from the Postojna region (Postojna–Planina Cave System, rainfall sample and surface streams) contain up to 444 MP particles per m^3. However, 32 samples taken from the Škocjan–Kačna–Jama 1 v Kanjaducah Cave System contain up to 60,000 MP particles per m^3, with the bulk of particles found in the sediment samples from Škocjan Caves – Kačna Cave System. Samples from Postojna region contained mostly PET, PU and PA polymers, with a minor inclusion of polymers of plastic sponge used for cleaning. Samples from Škocjan region contained mostly PP, PET and PE polymers, with some of PA and PU polymers. Sediment samples contained much less MP particles compared to water samples, which indicates fast transport through karst aquifer.

Keywords: caves, groundwater, contamination, aquifer, Postojna, Škocjan.

Izvleček UDK 551.435.8:504.5:691.175(497.47)
Lara Valentić, Peter Kozel, Tanja Pipan: Onesnaženje z mikroplastiko v ranljivem kraškem okolju: primer slovenskega klasičnega krasa
Z začetkom proizvodnje plastičnih polimerov pred več kot stoletjem se je pojavil pereč problem kopičenja plastičnih odpadkov v okolju. V zadnjih letih se problematika majhnih delcev plastike (mikroplastike, MP) pospešeno proučuje, kljub vsemu je malo znanega o količinah in vrstah MP v kraškem okolju. Namen te raziskave je bil odvzeti in analizirati vzorce iz kraških habitatov ter poskusiti določiti onesnaženje z mikroplastiko v kraških izvirih, ki se uporabljajo kot viri pitne vode. Od potencialnih virov onesnaženja smo vzorčili deževnico, izpust očiščene vode iz dveh komunalnih čistilnih naprav in izcedne vode iz smetišča. Analize polimerov potencialnih MP delcev smo opravili z metodo FTIR-ATR. Rezultati so pokazali, da osem vzorcev s postojnskega območja (Postojnsko-planinski jamski sistem, vzorec deževnice in vzorci površinskih voda) vsebuje do 444 MP delcev na m^3. Po drugi strani 32 odvzetih vzorcev iz jamskega sistema Škocjanske jame: Kačna jama ‑ Jama 1 v Kanjaducah vsebuje do 60.000 MP delcev na m^3, z najvišjo koncentracijo vlaken MP v jamskem sistemu Škocjanske jame: Kačna jama. Rezultati polimerne analize so pokazali, da vzorci s postojnskega območja vsebujejo največ PET, PU in PA polimere, z majhnim deležem polimerov, ki imajo podoben spekter kot plastična gobica za čiščenje. Vzorci s škocjanskega območja so vsebovali večinoma PP, PET in PE polimere, z manjšim deležem PA in PU polimerov. Vzorci sedimenta so vsebovali precej manj MP delcev kot vodni vzorci, kar kaže na hiter prenos onesnaženja skozi kraški vodonosnik.

Ključne besede: jame, podtalnica, onesnaženje, vodonosnik, Postojna, Škocjan.

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1. INTRODUCTION

Plastic pollution in the environment accounts for 60% to 80% of all human waste (Imhof et al., 2013), and it is the result of wear and tear of plastic products, combined with poor waste management in the past and present. The most abundant plastic polymers in the environment are polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS) and polyvinyl chloride (PVC), as they are the most produced plastic polymers (Fossi et al., 2012), but advances in analytical methods in recent years also allow the identification of other, less produced plastic polymers. The issue of (micro)plastic pollution and its impact on the environment is an important topic to be studied, as some plastic polymers can be highly toxic due to mutagenic and/or carcinogenic monomers, such as PVC. However, their toxicity may also be due to various additives and chemicals used in manufacturing that are released into the environment (Lithner et al., 2011), making it difficult to study their impact on the environment.

Microplastic particles are typically 1µm–5 mm in size, although the study of microplastic particles that are <50 µm is currently challenging due to limitations of analytical methods (Besseling et al., 2015; Horton et al., 2017). The presence of MP is one of the most topical issues globally, with studies ranging from human exposure to MP (e.g., Abbasi et al., 2019; Abbasi & Turner, 2021; Çobanoğlu et al., 2021), atmospheric transport (e.g., Beaurepaire et al., 2021), effects on organisms (e.g., Fabra et al., 2021), in deep-sea (e.g., Ferreira et al., 2022), in marine environment (Zhou et al., 2022), in wastewaters (e.g., Vuori & Ollikainen, 2022), to landfill leachates (e.g., Silva et al., 2021) and groundwater (e.g., Goeppert & Goldscheider, 2021). Studies on river MP litter in 57 rivers that discharge into the sea showed that MP particles were detected in 98.5% of samples, with a concentration of approximately 13–19 MP particles per 1000 m^3 (Schmidt et al., 2017). Additionally, the highest concentrations of MP particles in the environment are usually detected along densely populated coastlines. Therefore, it is reported that 500–10200 MP particles per m^3 are present in southeastern coast of Korea (Wang et al., 2019), and since 49% of all produced plastics can float on the water surface they can reach even the most remote areas (Botterell et al., 2022; Huang et al., 2022). For example, the concentration in the Ross Sea (Antarctica) is reported to be 1.18 MP particles per m^3 (Cincinelli et al., 2017). So it is not farfetched to assume that bigger quantities of MP could also be present in the karst underground and karst aquifers.

Karst aquifers are carbonate rocks with channels and fissures of different sizes, which are capable of storing relatively large quantities of groundwater due to high rock porosity. They can be easily contaminated by human activities and surface pollution (at least in Slovenia; Ravbar, 2007; Ravbar & Kovačič, 2015), as the carbonate bedrock is usually covered only by a thin layer of soil, water drains rapidly through numerous channels and fissures, and point recharge of water occurs through sinkholes (Ford & Williams, 2007; White, 2019). Consequently, pollutants can be translocated rapidly and deeply into the underground (Vižintin et al., 2018). Moreover, percolating water and sinking rivers are the main aquifers' recharge pathways (Goldscheider & Drew, 2007; Petrič et al., 2011). Sinking rivers can also flow from the non-karst edge and sink on contact with karst in well-developed karst systems (Petrič et al., 2011). Therefore, pollution can be transferred over even greater distances. Another important characteristic of karst aquifers is bifurcation of sinking rivers and percolating water (= water flows into different catchment areas), which in turn has an important effect on the transport of pollution under different hydrological conditions (Kovačič & Ravbar, 2013).

The most common contamination sources of karst waters are urban, household and industrial wastewaters, untreated rainwater runoff from roads and built surfaces, agricultural contaminants (Culver et al., 2012; Kovačič & Ravbar, 2013; Cheung & Fok, 2016; Talvitie et al., 2017) and leachate from legal and illegal waste disposal sites (landfills; Silva et al., 2021). Landfill leachates are complex solutions of nitrates, phosphates and sulphates that accelerate the dissolution of the carbonate rocks underneath landfill site, meaning that infiltration of other pollutants is much easier (Kogovšek, 2011). In addition, many illegal landfills are located inside caves and vertical shafts, with approximately 10% of caves in Slovenian lowlands being polluted (Culver et al., 2012). Another threat lies in the spills of greater quantities of hazardous and toxic substances during accidents (Culver et al., 2012).

Recently, another major problem has been detected in karst – MP contamination (Valentić, 2018; Panno et al., 2019; Balestra & Bellopede, 2022). Research into MP in karst environments and in karst aquifers is still in its infancy, so suitable sampling methods and protocols have yet to be developed. The main pathways for MP transport in karst environments are percolating water and sinking rivers – bringing MP directly or indirectly from the surface, where they are deposited by precipitation, agricultural pollution and pollution of dolines, caves, and intermittent lakes. According to the few existing preliminary studies, the karst underground environment contains predominately MP fibres (Valentić, 2018; Panno et al., 2019; Balestra & Bellopede, 2022), with study of Valentić (2018) indicating fewer MP in sediment samples than in water samples, showing a correlation between the low density of MP and rapid transport of such particles through the karst aquifer. However, the study by Balestra and Bellopede (2022) focused exclusively on cave sediment samples and found relatively high concentrations of MP in their samples. Panno et al. (2019) also found a correlation between pharmaceutical and personal care product pollution and MP pollution. It is important to understand karst pollution with MP because karst covers about 15% of the global land surface and more than a fifth in Europe alone (Chen et al., 2017, Goldscheider et al., 2020), even though globally only around 4% of drinking water is obtained from karst sources (Stevanović, 2019).

Although groundwater ecosystems are increasingly recognized for their ecological and socio-economic value (Bonacci et al., 2009; Cantonati et al., 2020), they have not yet been adequately documented and investigated with regard to MP pollution. The aim of this study was to fill this gap and better assess MP pollution by collecting and analysing samples from various karst habitats, and to try to determine the extent of pollution of karst springs, some of which are used as drinking water sources. Therefore, we devised the following research questions:

(1) Although research on MP in karst environments is still very sparse, MP can be found in the entire karst environment. We expect peak concentrations to occur primarily in tourist caves or tourist parts of caves. To what extent are these sites more polluted with MP than sites not exposed to tourist activities? To what extent does the amount of MP depend on cave management?

(2) Activities in the catchment area are important and have an impact on the quantity of MP found in certain karst habitats. Is the Škocjan region, which is in direct contact with the Reka River, more polluted with MP due to a factory producing household plastic products in Ilirska Bistrica? Can the determination of MP polymers present in different karst habitats help us to determine the sources of pollution in the karst environment?

2. MATERIALS AND METHODS

2.1 STUDY AREA

The study was conducted in Slovenia, with two sampling sites in Italy. In Slovenia, karst covers almost half of the country and karst aquifers provide drinking water to more than 50% of the population (Petrič et al., 2011). Samples were taken in two karst areas (Figure 1) – in

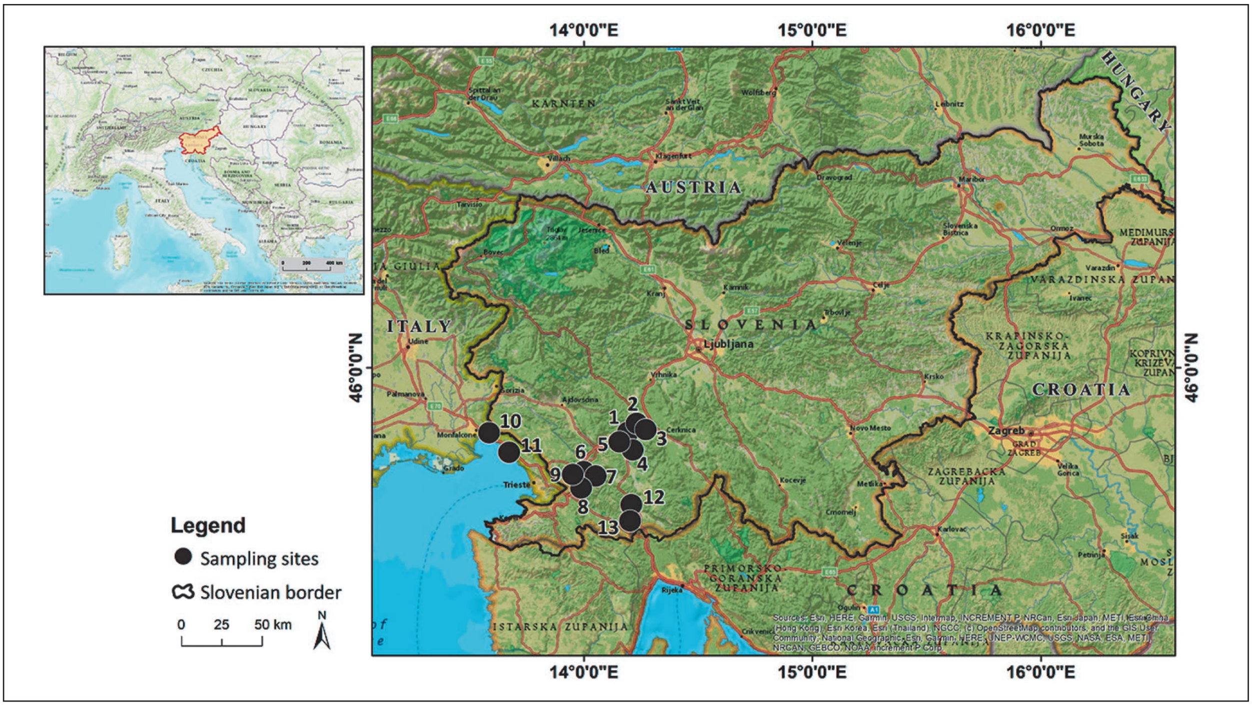

Figure 1: Map of Slovenia with research area and sampling locations. Numbers 1–5 represent sampling points connected with Pivka (Postojna area): 1 = Postojna Cave System, 2 = Planina Cave, 3 = Malni spring, 4 = wastewater treatment plant Postojna, 5 = rainfall samples. Numbers 6–13 represent sampling points in Škocjan area: 6 = Škocjan caves, 7 = surface samples of Reka, 8 = Kačna cave, 9 = cave Jama 1 v Kanjaducah, 10 = Timavo springs of Reka, 11 = Brojenca spring of Reka, 12 = wastewater treatment plant Ilirska Bistrica and landfill discharge Ilirska Bistrica, 13 = artificial Lake Mola (both maps are obtained from OpenStreetMaps using ArcGIS programme and were done by M. Năpăruș-Aljančič).

the Postojna and Škocjan regions. Two sinking rivers (the Pivka River and the Reka River, hereafter referred to as Pivka and Reka) with their wider catchments and two adjacent large cave systems (Postojna–Planina Cave System and Škocjan–Kačna–Jama 1 v Kanjaducah Cave System) associated with each sinking river were sampled. Other water and sediment samples were collected from: 1) Malni karst spring in the Postojna region, which is the main drinking water supply; 2) Timavo springs near Trieste, Italy, where the Reka re-emerges on the surface as the Timavo River; 3) Brojenca karst spring, located near Timavo springs; 4) landfill leachate discharge in the Reka catchment area; 5) discharges from wastewater treatment plants in Postojna and Ilirska Bistrica; 6) artificial Lake Mola; and 7) rainwater. In total, 58 water samples and 29 sediment samples were analysed, in order to determine important pathways of MP transport from terrestrial and aquatic environments in the karst ecosystem, as well as the dynamic of MP transport in the environment.

2.2 SAMPLING METHODS

Sampling methods were adapted to the specifics of each sampling site, but were generally as described below. All sampling was conducted from January 2017 through March 2018, with each sampling site (except rainwater) sampled once. More detailed data are presented in Table 1 and summarised in Figure 2. Rainwater was collected in a generic PE (polyethylene) sturdy container (35 x 35 x 35 cm size) that was set up during the rain event and removed immediately after. Two rain events lasted 4–6 h, while the rest were heavy downpours that lasted 3–4 days at a time. The container was cleaned beforehand with milliQ water and installed away from trees, gutters or litter. Precipitation samples in the town of Postojna were collected on a remote meadow, while samples in Škocjan were collected on a lawn behind the tourist information centre in the Škocjan Caves System regional park.

Water samples from surface streams and inside the caves were taken with a 60 μm mesh size sampling net, with a trap attached at the lower part of the net, and were sampled on the surface of the streams. Water from small puddles and rimstone pools was sampled either with the sampling net described above or using methods similar to those used for sampling epikarst fauna in drips and shallow pools (Pipan, 2005). The sampling net and PE plastic container for shallow puddles were cleaned beforehand with either milliQ water or washed downstream to prevent cross contamination of the samples. Water samples were then immediately transferred into clean glass bottles, that were also rinsed with milliQ water. Cross-contamination from any plastic material used was eliminated with analysis of all the plastic polymers used in this process.

Drips inside the caves were sampled with methods similar to those used for sampling an epikarst fauna in percolating water (Pipan, 2005). In general, the volume of filtered water ranged from 0.1 L (in shallow puddles) to almost 17 m^3 (wastewater treatment plant discharge) and depended on the velocity of the water currents (streams) and the size of puddles and rimstone pools.

To get representative samples of cave sediments, we dug up to 5 cm layers of sediment, taking several subsamples from each sampling site and regarded them as one composite sample. Sampling sites were chosen based on accessibility and where there was no water above the sediment, to prevent washing away of the MP with water. All subsamples were collected with a metal spatula and stored in clean containers. Both were cleaned with milliQ water before use and between sampling sites. The size of the sampling plot, the number of subsamples and

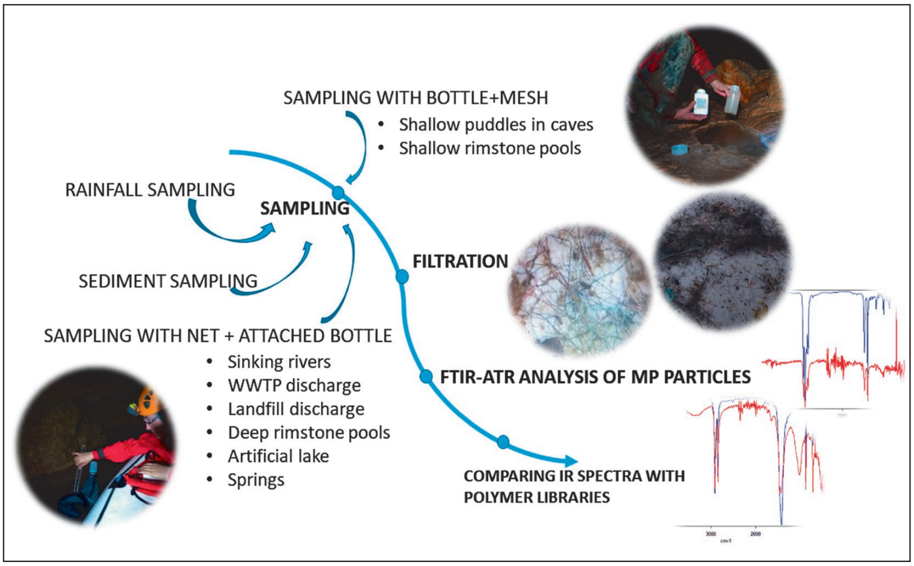

Figure 2: Schematic representation of field sampling and laboratory methodology for microplastic analyses. WWTP = wastewater treatment plant.

quantity of the sediment sampled varied, depending on the characteristics of each sampling site. On each sampling site, we scooped between 0.5 and 1 kg of sediment. Sediment samples inside the Postojna Cave System were taken close to the tourist path or close to the cave railway tracks.

Throughout the study we followed standard procedure (see Palatinus et al., 2015) to prevent sample contamination. We also ran procedural blanks with 100 mL milliQ water that were treated the same as the water samples, and measured the plastic spectra of the plastic equipment we used to determine possible contamination. The blank samples did not contain microplastic particles/fibres.

2.3 LABORATORY ANALYSIS

In the laboratory, all samples were filtered through paper filters with 12–25 µm mesh size (manufacturer IDL GMBH & CO., Germany) and covered with aluminium foil (cleaned with acetone) to prevent deposition of MP from ambient air. After filtrations, filters were stored in aluminium foil that was cleaned with acetone and a paper towel (hereafter referred to as clean foil). All laboratory equipment was cleaned beforehand with acetone and covered with clean foil. Water samples were filtered without any prior sample preparation, while sediment samples were first transferred to clean foil and dried overnight at 105 °C, as the wet sediment could not be sieved without major losses of potential MP (especially clay sediments from caves). After drying, the sediment was sieved through a 2 mm sieve to remove stones and particles of organic material (leaves, twigs, etc.). The material that remained on the sieve was visually examined for MP particles, while the rest was extracted based on the protocol of Palatinus et al. (2015). The only change we made to the protocol was letting the sediment samples settle for six minutes.

After filtrations, filters were visually inspected in closed, clean glass petri dishes under a Bresser stereomicroscope, using 4x magnification. Potential MP particles were chosen based on their colour and shape (Hidalgo-Ruz et al., 2012) and were manually transferred using tweezers onto an FTIR-ATR instrument to measure the IR spectrum of each particle. A Perkin Elmer FT-IR Spectrum 100 spectrometer was used, coupled with PIKE Technologies GladiATR that has a diamond crystal. Samples were measured in a spectral range of 4000 to 30 cm^{-1} and accuracy of +/- 0.5% of set point (Pike Technologies, 2018).

The results were compared with spectra of known polymer types of plastic – PVC (polyvinyl chloride), PP (polypropylene), PS (polystyrene), PET (polyethylene terephthalate) and PE (polyethylene), that were gathered in our database by measuring conventional plastic products that could be potential sources of MP in the environment. Later, to confirm our results, measured spectra were also compared with the licenced KIMW FTIR-ATR polymer libraries of plastic and plastic additives spectra for a Lumos II FTIR-ATR microscope.

2.4 STATISTICAL ANALYSIS AND CALCULATIONS

The number of MP particles per m^3 of water sample was calculated by dividing the number of found MP particles by the volume of the sample. The number of MP particles per m^3 of sediment sample was calculated with extrapolating the number of MP particles found in 150 mL of sediment sample (three subsamples of 50 mL) to 1 L of sediment and consequently, to m^3.

We wanted to verify whether the Postojna and Škocjan regions are equally contaminated with MP, so we analysed samples from both regions for differences in the number of plastic fibres per m^3 of sample. Due to non-normally distributed data, as indicated by the Shapiro-Wilk Test (Postojna region: $W = 0.40$, $p < 0.001$; Škocjan region: $W = 0.21$, $p < 0.001$), we applied the Mann-Whitney U test. The Spearman rank correlation coefficient (rs) was calculated to examine the correlation between the volume of filtered water and number of plastic particles per m^3 of sample in both regions. Statistical analysis was done with the programme PAST (Hammer et al., 2001).

3. RESULTS

Out of 20 water samples that contained MP particles, 17 were cave samples that were taken from different shallow pools where water is retained for a long period of time (from weeks to decades, depending on the sampling site; Kluge et al., 2010; Kogovšek, 2010). The majority of samples with confirmed MP particles contained fibres, with only two particles having a sponge-like structure. Altogether, we analysed 44 potential MP particles in water and sediment samples from Postojna region, and 159 potential MP particles in water and sediment samples from Škocjan region. Out of that, 39% and 49% were successfully identified as MP in Postojna and Škocjan region

Table 1: Summarised sampling data with sampling locations, dates, volumes of filtered water and number of samples. The number of microplastic particles was obtained using FTIR-ATR analysis, while the number of microplastic particles per L is calculated as described in the text. WWTP = wastewater treatment plant.

	Sampling location	**Type of samples**	**Sampling date**	**Total volume of filtered water or sediment (m^3)**	**Number of samples**	**Total number of microplastic particles**	**Total number of microplastic particles/ m^3 (mean±StD)**
Postojna region	Rainwater	water	Jun, Sep 2017	0.0045	3	2	444.44 ± 0.77
	WWTP	water	Jan 2018	0.310	2	0	0.00
	Pivka	water	May 2017, Jan 2018	0.735	2	0	0.00
	surface stream	water	Jan 2018	0.700	3	1	1.43 ± <0.01
	surface stream	sediment	Jan 2018	0.00015	1	0	0.00
	Malni spring	water	Jan 2018	0.400	1	0	0.00
	Malni spring	sediment	Jan 2018	0.00015	1	0	0.00
	Planina cave system	water	Jan 2018	0.300	3	0	0.00
	Planina cave system	sediment	Jan 2018	0.00015	1	0	0.00
	Postojna cave system	water	Dec 2017, Feb 2018	14	14	14	1.00 ± 1.48
	Postojna cave system	sediment	Feb 2018	0.00015	6	0	0.00
Škocjan region	Rainwater	water	Jan, Mar 2018	0.00034	3	0	0.00
	Landfill leachate	water	Jan 2018	0.105	1	0	0.00
	Landfill leachate	sediment	Jan 2018	0.00015	2	0	0.00
	Artificial lake Mola	water	Jan 2018	0.105	1	0	0.00
	Artificial lake Mola	sediment	Jan 2018	0.00015	1	0	0.00
	Reka	water	Jan 2018	0.300	1	0	0.00
	Reka	sediment	Jan 2018	0.00015	1	0	0.00
	WWTP	water	Jan 2018	20	2	0	0.00
	Timava springs	water	Jan 2018	0.105	4	1	9.52 ± <0.01
	Timava springs	sediment	Jan 2018	0.00015	4	0	0.00
	Brojenca spring	water	Jan 2018	0.105	1	0	0.00
	Brojenca spring	sediment	Jan 2018	0.00015	1	0	0.00
	Kačna cave	water	Oct 2017	0.180	5	3	16.67 ± 0.01
	Kačna cave	sediment	Oct 2017	0.00015	4	1	6666.67 ± 3.33
	Jama 1 v Kanjaducah	water	Nov 2017	0.108	2	0	0
	Jama 1 v Kanjaducah	sediment	Nov 2017	0.00015	4	0	0
	Škocjan Caves system	water	Jan, Mar, Oct, Nov 2017	6.7	13	64	9.55 ± 16.64
	Škocjan Caves system	sediment	Jul, Nov 2017, Jan 2018	0.00015	4	9	60000.00 ± 17.53

Table 2: Summarised microplastic spectra that were found in samples in different locations.

	Sampling location	Type of samples	Microplastic polymers found in samples
Postojna region	Rainwater	water	other*
	surface stream	water	PA
	Planina cave system	water	no microplastic
	Postojna cave system	water	PET, PU, PARA
Škocjan region	Rainwater	water	no MP
	Timava springs	water	PE/EVA
	Timava springs	sediment	no microplastic
	Kačna cave	water	PET
	Kačna cave	sediment	PP
	Škocjan Caves system	water	majority PP and PET, some PE, PE/EVA, EPDM, PU, PMMA, PA, other*
	Škocjan Caves system	sediment	PE, PU, PET

PET = polyethylene terephthalate, PARA = polyacrylamide, PE = polyethylene, PP = polypropylene, PU = polyurethane, PA = polyamide, EVA = ethylene-vinyl acetate, EPDM = ethylene propylene diene rubber, PMMA = polymethyl methacrylate. Other = PBT (polybutylene terephthalate), PTFE (Polytetrafluoroethylene), copolyester, TPV (thermoplastic vulcanizates), EBA (ethylene butyl acrylate), fibres from dishwashing sponge. Spectra for polymers in other* category are shown in Supplementary Information (Appendix A; Figures A.1-A.13). The spectra for all samples were first compared to the database we constructed out of commonly accessible plastic products, but were later double-checked with certified library of spectra inside Lumos II.*

respectfully – the exact quantity of confirmed MP particles in the samples is summarised in Table 1.

At the time of research, rainfall samples were expected to be blank and not contain any MP, since the sampling sites were chosen far away from any direct source of pollution. However, one sample was found to contain microplastics (Table 1). This rainfall sample was from Postojna and was collected over a longer period of heavy precipitation that lasted for several days. Since the campaign was conducted, different studies of rainfall and air confirmed that both can be a very important source of pollution, especially in remote areas (e.g., Beaurepaire et al., 2021).

Postojna region had eight water samples with confirmed microplastics (out of 20), and none of the sediment samples contained microplastic particles. On the other hand, Škocjan region had confirmed microplastics in 12 water samples out of 20, and in all four sediment samples.

It was found that samples from Postojna region contained MP with a confirmed PET polymer spectrum (Figure 3), but in some cases fibres/particles were PA (polyamide), PU (polyurethane), PARA (polyacrylamide) or had a polymer spectra similar to that of a plastic sponge for household cleaning (Table 2). A lot of times, measured particles in all samples from both regions, had spectra that were indicating natural origin (e.g., cotton).

Figure 3 shows some results for spectra of measured fibres, including only those that are the most representative. The rest of the spectra are shown in the Supplementary Information (Appendix A). Samples from Škocjan region contained mostly MP with either PP or PET polymer spectrum (Figures 3a, 3b). Other represented polymers include PE (Figure 3c), PA, EBA, EVA and others. One sample from Škocjan Caves System contained the thickest fibres (0.67 mm wide), that were easily separated for analysis. It contained fibres that had mostly PP spectrum, with few fibres that were PET spectrum. Other samples (from both regions)

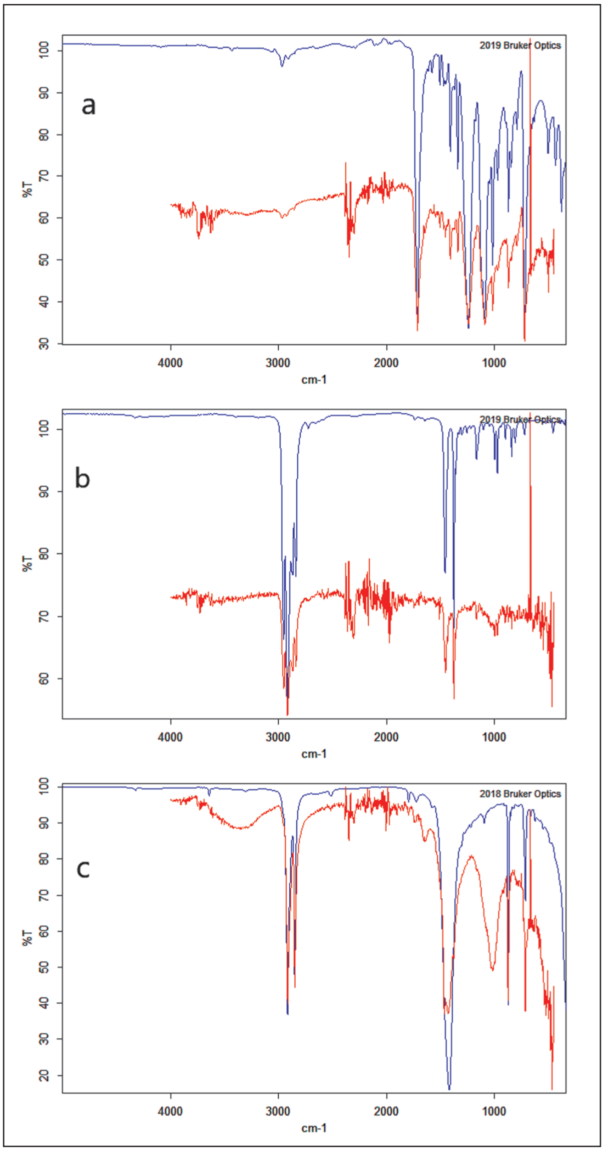

Figure 3: Three results of measured fibres. The spectra of fibres are represented in red, while those of plastic polymers are in blue. a) PET polymer that was found in a sample from the Postojna Cave System. b) PP spectrum found in a sample from Škocjan Caves. c) PE spectrum from a sample from Škocjan Caves.

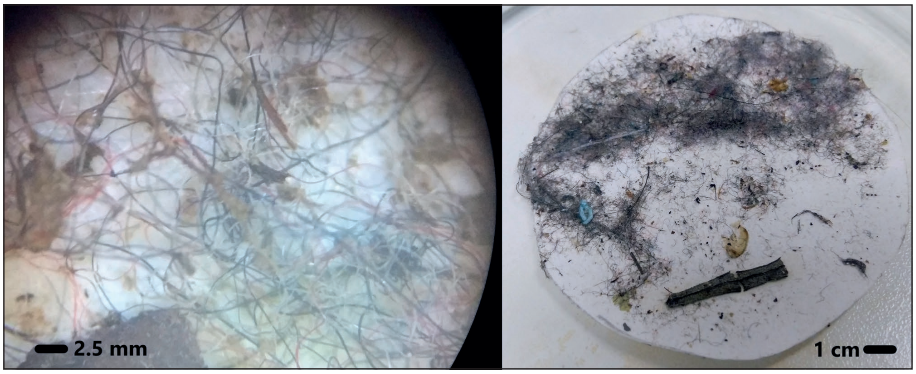

Figure 4: Example of a challenging sample from the Postojna Cave System. On the left side is a filtered sample under a stereomicroscope (4x magnification). The image on the right side is a complete sample.

contained thinner and shorter MP fibres (less than 0.067 mm wide and approx. 1 mm long).

Figure 4 shows an example of samples that were very challenging for analysis, as the filter contained an immense number of firmly interlaced and thin fibres. It was, therefore, hard to separate them manually (Figure 4).

During the analysis, we found out that six minutes of settling time for the sediments was not enough, especially for the clay sediments from caves. Moreover, we found that density separation for sediments that contained a lot of organic material is not an adequate procedure, as the filters still contained a lot of organic material that obscured visual examination for potential MP particles.

There were no statistically significant differences in the number of plastic fibres per m^3 of sample between Postojna and Škocjan regions (U = 395, p = 0.75). We found a low negative correlation between the volume of filtered water and the number of plastic fibres per m^3 of sample for samples from the Postojna region (rs = −0.38, p = 0.06), and a moderate positive correlation for samples from the Škocjan region (rs = 0.54, p = 0.11), but neither correlation was statistically significant.

4. DISCUSSION

Karst systems contain many natural resources, host a high degree of biodiversity and deliver valuable ecosystem services (Culver & Pipan, 2019; Goldscheider, 2019), but unfortunately our findings showed that karst systems are not an exception to MP contamination, as was also shown by Valentič (2018), Panno et al. (2019) and Balestra and Bellopede (2022). Analyses of water and sediment samples in our study partly confirmed research question (1) – MP is present in both karst regions in Slovenia (Postojna and Škocjan, see Tables 1 and 2), with the highest concentrations of MP found in the tourist parts of both cave systems. Although we did not find large quantities of MP in individual samples, MP were present in approximately one third of the samples (28%). When correlating data to number of particles per m^3, we can see that the most polluted samples were rainfall in Postojna region and sediment samples from cave system Škocjan Caves – Kačna Cave, which is in agreement with more recent studies. A majority of the confirmed MP in our study were fibres and not pellets or other irregularly shaped particles, as are mostly found in other environments, especially marine environments (e.g., Cheung & Fok, 2016).

Furthermore, the impact of cave visitors on the quantity of MP is an important factor, as the number of found particles was higher in rimstone pools next to the tourist paths, but we were unable to determine all seen particles. That was especially evident in samples from Postojna Cave, that has a long history of cave tourism and is Slovenia's most famous show cave – visited by more than 38 million visitors to date (Šebela, 2019). The tourist path of the Postojna Cave is never flooded (Arsenović, 2007), and therefore MP pollution is a direct result of either cave visitors, infiltration with percolating water (Valentić, 2018), natural cave ventilation (Gams, 1974; Šebela, 2019) or a result of all of the above. Since MP pollution in non-tourist parts of the caves was minimal compared to the touristic parts, we can assume that percolating water and natural cave ventilation are not important contributors to the pollution. That was also the case in Škocjan Caves System, where the tourist path is 90 m higher than the Reka riverbed and, therefore, rarely flooded. This further confirms an important role of cave tourism in pollution with MP, even though the Škocjan Caves System has significantly fewer visitors than Postojna Cave, and with Kačna Cave and Jama 1 v Kanjaducah being accessible only to professional cavers. However, we need to take into consideration that rimstone pools in the Postojna Cave System are regularly cleaned (Mulec, 2014, 2019) with pressurised water by the management company, and therefore the quantity of MP in the Postojna–Planina Cave System could be substantially higher than found in this study.

Rainfall samples collected in Postojna and Škocjan regions also contained MP which partly confirms research question (2) – there were plastic fibres found in those samples (Tables 1, 2), but also fibres that were natural, therefore, activities in the catchment can have an impact also in distant habitats. As Li et al. (2018) described, these were airborne MP that are present also in remote areas. Recently, studies of MP in rainfall and the atmosphere are becoming more common, especially when doing comparisons with remote areas. Even though only a handful of studies have been conducted since 2015 (i.e., Chen et al., 2020), all have found fibres in their samples, as was also the case in our study. Furthermore, Allen et al. (2019) confirmed that MP can be transported over long distances by the wind.

Sediment samples contained only a few MP particles and/or fibres, compared to water samples, although the Škocjan Caves System is especially heavily polluted with macroplastic litter that is a runoff from the town of Ilirska Bistrica onwards (Valentić, 2018). But when the data are computed per m^3, the results are drastically different – the most polluted are sediments from Škocjan Cave System and from Kačna Cave System, which confirms our expectations and research question (2). Ilirska Bistrica town can present an additional threat of MP pollution as it has a factory which produces plastic packaging and other common household plastic products. In addition, Reka is a torrential river that frequently floods caves, therefore macroplastic litter brought inside is likely to be broken into smaller MP pieces and deposited in the sediment along the river's course through the cave. We have to take into consideration, that sampling in Škocjan Caves System was conducted during a dry period because the underground canyon is inaccessible during high water periods. Therefore, it would be interesting to see possible differences in results if we sampled for longer time, before and after floods, as higher water levels can easily transport MP deeper into the cave system.

The discrepancy for sediment samples from Postojna region could be because we sampled only recent sediment after floods and it is possible that during flooding MP particles did not have time to settle but were rather rapidly transported further. Other sampling sites for sediments were close to railway tracks so we would expect more MP pollution. It is possible that since the sediment near railway tracks is heavily compacted, the MP pollution is transported with cave ventilation further in the cave. This further reflects fast MP flow through the karst underground, which was also confirmed in the latest study by Goeppert and Goldscheider (2021). They confirmed with a tracing experiment in an alluvial aquifer that MP spheres travel much faster than solutes, and therefore MP could be deposited in karst sources of drinking water (Valentić, 2018; Panno et al., 2019). With our results we cannot either confirm or deny this claim, as we would need a different sampling method for springs than the one we used.

During the analysis, we found out that the colour of the fibres is not the most reliable indication of the potential MP – many times transparent, blue, red and black fibres had spectra similar to cotton or some other natural polymers. Therefore, we need to eliminate subjective decisions by analysing all fibres/particles with FTIR-ATR microscopy. With this, we would eliminate the manual transfer of fibres/particles from filters to FTIR and the possibility of losing the particles or cross contamination. Since FTIR-ATR microscopy is a non-destructive analytical method (Ismail et al., 1997), it is also possible to conduct further analysis of particles if needed.

In our study, we found mostly fibres, therefore we can assume that the major source of pollution is clothing – either from cave tourism or from households. But the results of plastic spectra we have measured do not give us exact answer so we cannot confirm research question (2). It is well known that PA (with PARA as one type of PA) is mostly used in clothing, so the fibres with these spectra are expected. But we are not sure what are the sources of fibres that have i.e., PET spectra, as PET is mostly used in packaging. PU is a resin, so it could be possible to have some other material produced as fibres and then coated it in PU.

On average, we found 0.56 MP particles/m^3 in the Postojna region, and 2.78 MP particles/m^3 in the Škocjan region, so we can conclude that the Škocjan region is more polluted than the Postojna region. Although we need to take into account the fact that samples in the Škocjan region are distributed over a larger catchment area compared to the Postojna region (approximately 205 km^2 for the Škocjan region vs. 6 km^2 for the Postojna region; ARSO, 2019), we believe that the greater MP pollution in **Škocjan** region might be due to certain activities in the catchment area – Reka is regularly polluted with macroplastic litter (Valentić, 2018) and Ilirska Bistrica has a factory producing household plastic products, which confirms research question (2).

In future work, a standard sampling protocol and long-term monitoring in different hydrological conditions for MP in karst underground environments has to be developed. During our research, it was found that filtration of sediments rich in clay or other organic matter using the protocol in Palatinus et al. (2015) is a robust method which, however, does not bring satisfactory results. There is a possibility that MP can concentrate in the underground during dry periods, as was confirmed with numerous studies on water soluble pollutants in the karst (i.e., Gabrovšek & Turk, 2011; Kogovšek, 2011; Petrič et al., 2011). Thus, it is necessary to develop effective methods for assessing the quantities of microplastics in terrestrial and aquatic karst environments. We expect that using FTIR-ATR microscopy for polymer analyses would greatly improve the quality of data as it will eliminate the need for manual transfer and reduce the subjective error in determining potential MP particles. This would help us better understand the transport routes in karst environments and ease the determination of pollution sources, which could help us to limit the pollution of the karst underground with MP in the future. The transport dynamics and pathways of the MP waste through karst terrestrial and aquatic settings are unfortunately poorly studied, and therefore remain a mystery.

5. CONCLUSIONS

We conducted a study on the presence of MP in two karst regions in Slovenia and in Italy, with a wide range of samples to determine possible pollution sources and transport routes. We found MP in 28% of samples, with the majority of polluted samples being water samples – in 20 samples versus four sediment samples – which indicates fast transport of MP pollution through the karst system. We partially confirmed research question (1): a) the highest concentrations of MP found are indeed in tourist parts of the caves; b) tourism in the caves is an important source of pollution, as the majority of MP were found next to the tourist paths; and c) cave management plays an important role of MP pollution as even though Postojna Cave System has much higher number of visitors per year, we found less MP pollution than in Škocjan Cave System. This could be due to regular cleaning of rimstone pools in Postojna Cave System, whereas Škocjan Cave System does not. Research question (2) was also partially confirmed: a) Škocjan region is heavily polluted with macroplastics in the wider catchment area and we found more MP in samples from Škocajn Cave System compared to other samples in the same region. This can be due to heavy pollution of Škocjan Cave System with macroplastic litter that Reka breaks down and transports it further in karst massif. Macroplastic litter could be a consequence of plastic factory in Ilirska Bistrica, but there are not enough data for this conclusion. b) Determination of plastic polymers does not necessarily help us in identifying pollution sources. We found mainly fibres and only a few irregularly shaped MP particles, which could indicate clothing as the major source of pollution (either from visitors in caves or household) but we could not irrefutably identify other sources of pollution. In Postojna region, we found mostly PET polymers, with some PU, PARA and PA polymers, while in Škocjan region we found mostly PP and PET polymers, with some PE, PA and PU polymers. This are rather conflicting results as PET and PP are not usually used in clothing but rather in packaging. We also need to take into consideration, that MP particles can be transported deep into karst massif with high water levels, therefore it is possible MP particles concentrate in the underground and are flushed out during flooding events.

AUTHOR CONTRIBUTIONS

Lara Valentić and Tanja Pipan equally conceived the presented idea, designed the research, conducted fieldwork and analysed the data, and contributed equally to the writing of the manuscript. Peter Kozel conducted statistical analyses and contributed to the final version of the manuscript.

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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.

APPENDIX A.

Supplementary data include some representative spectra of plastic fibres from the research study described in this article. Spectra were obtained by comparison of licenced KIMW FTIR-ATR polymer libraries of plastic and plastic additives spectra for a Lumos II FTIR-ATR microscope.

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