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DISTRIBUTION AND ABUNDANCE OF MICROPLASTICS IN UNDERGROUND RIVERS IN THE SOUTH MALANG KARST AREA: FIRST EVIDENCE IN INDONESIA

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Abstract

Introduction: *The presence of microplastics in the environment increases the diversity of types of pollutants in waters, including clean water sources on the surface and underground. Karst areas have unique hydrological characteristics, with cracks and fissures between the rocks that can be potential routes for the transport and accumulation of microplastics in underground river flows. In this study, we want to know the distribution and abundance of microplastics in underground rivers in the karst area of South Malang, Indonesia.* ***Methods:*** *Samples were collected purposively from underground river of Lowo, Banyu and Sengik. Microplastics were prepared using a diluted solvent of 30% H_2SO_4 and 30% H_2O_2. The sediment sample was dried and then filtered twice using 300-mesh size nylon filters until microplastic particles accumulated.* ***Results and Discussion:*** *Microplastics were detected in all water samples, with an average abundance ranging from 1.8 to 2.3 particles per liter. Fibers were the dominant microplastic type, followed by fragments and films, while the color distribution includes blue, white, red, yellow, black, green, pink, and brown.* ***Conclusion:*** *Microplastic contamination has been found in underground river flows in the karst area of South Malang, Indonesia. Karst soil, traditionally viewed as a natural filter, is not impervious to plastic pollution. This suggests significantly higher surface contamination than previously assumed. Therefore, reducing surface pollution is essential to safeguard the precious quality of underground aquifers and protect public health above.*

INTRODUCTION

Environmental problems continue to increase yearly with increasing production of raw materials and increasingly high levels of public consumption, causing increased water environmental pollution, both in quantity and quality. Even though the need for clean water continues to increase along with the increase in population, this is stated in the sustainable development goals initiated by WHO, where the target to be achieved is that 100% of the world's population can access clean water by 2030 (1). The water pollution that people need to be aware of today is microplastics. Microplastics are plastic fragments from primary and secondary sources with a particle size of less than 5 mm (2).

The statement that microplastics can affect human health is still being debated. However, microplastics are non-biodegradable elements, so they have potential health risks if exposed to humans because microplastics are xenobiotic (foreign material to the body). Microplastic contamination in the environment continues to increase, so serious attention is needed, considering that Indonesia is one of the world's second-largest producers of unmanaged plastic waste (3). Apart from that, several studies from various experts have found that traces of microplastic contamination have been found in the middle of the sea (4), in polar regions (5), in places rarely visited by humans (6) and also in karst areas (7).

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Karst is an area that has a dry and rocky surface consisting of limestone rock. Karst aquifers are karst landscapes that store extraordinarily abundant water reserves. Aquifers are formed from rock formations where the solvent is blocked due to the resistant type of rock. A study revealed that karst aquifers have the potential for vast water reserves to meet the needs of a quarter of the world's population (8). The karst aquifer system is complex and complicated, so if simplified, the karst aquifer is the water in the underground Cave and the karst area above it, or it can also be called an underground river in the Cave.

More than 20% of the land surface on Earth is a karst layer. Almost every large island in Indonesia has a karst area, such as the islands of Sumatra, Java, Sulawesi and Papua. On the island of Java, especially in East Java, almost all the coastlines are karst areas, one of which is Malang Regency. South Malang Karst is part of the karst area in Indonesia among dozens of designated karst areas (9).

According to a recent analysis by Balestra (7), various forms of microplastic particles were obtained from underground rivers in the Bossea Karst region in Italy, including microfiber (70%), fibre (14%), microfragment (14%), microfilm and fragments respectively (0.3%), as well as a few microbeads. Most of the water systems in karst areas come from infiltration of the soil above them, so the source of particle contamination is suspected to be microplastic either directly or indirectly. Microplastic pollution in groundwater can also come from stormwater (runoff channels during the rainy season) or street dust (10).

While microplastic abundance serves as a crucial metric, a comprehensive understanding of this pervasive pollutant demands delving deeper into its composition and size distribution. In determining the level of microplastic pollution, abundance is the main element for understanding the presence of pollutants that contaminate the environment. Apart from that, composition and size distribution are also important points in a study of microplastics. Studying the composition of microplastic in this karst area could lead us to predict the origin of the microplastic that indicating a unique source of pollution and degradation process, which is different compared to other areas. By mapping the interrelationships of size distribution, composition, and location, we will gain a deeper understanding of transport pathways, local variations in abundance, and the specific threats posed by microplastics in different environmental contexts. Furthermore, research findings show that microplastics have been found in human lungs (11-12), semen (13) and even placentas (14), it is raising concern about potential heatlh consequences.

Malang Regency is the district with the second largest population in East Java, with 2,654,448 as of 2020 (15). Geographically, the area is very diverse, from mountains to oceans. The Donomulyo Sub-district, located at the southern tip of Malang Regency, has quite a complex landscape. Based on the interviews with local people, information was obtained that there is an underground river in Lowo Cave, Banyu Cave and Sengik Cave in Donomulyo District, Malang Regency, which the local community can use to meet. Therefore, this research was conducted to detect the presence of microplastic particles based on the abundance, type and color in water and sediment in underground rivers in caves in the karst area of South Malang Regency. In addition, with the enermous potential that underground rivers have, to maintain their sustainability it is necessary to monitor pollution levels, especially microplastic pollution.

METHODS

The method used is a non-parametric analysis method with a quantitative approach to see and describe the object under study according to field conditions and draw conclusions about this with numbers according to the phenomena seen in the research. As well as measuring differences in abundance between research sample points.

The research was conducted from May 2022 to July 2022. The sampling locations were at Lowo Cave, Banyu Cave and Sengik Cave. Sample identification was carried out in the Ecology laboratory of the integrated laboratory at UIN Sunan Ampel Surabaya.

Sampling of underground river water and sediment in the Cave was carried out in the Donomulyo District area, Malang Regency, East Java. This area is a karst area that is part of the Nampol karst formation, formed during the middle Miocene in the geological period that occurred around 11 to 16 million years ago. Samples were taken at the surface at the average speed of river water flow, and sediment below.

Sampling was carried out at 2 points in each Cave. The following is the location for sampling underground river water in a cave in South Malang:

1. Lowo Cave: at coordinates (8°22'28" S, 112°27'38" E)
2. Banyu Cave: at coordinates (8°20'37.3"S 112°25'11.6" E)
3. Sengik Cave: at coordinates (8°21'58.7"S 112°23'49.3" E)

Sample Preparation

To collect traces of microplastic contamination, 500 mL water samples were taken from underground river flows in three caves and filtered using 300-mesh size nylon filters. After a few minutes, the filter was removed, and then distilled water was dripped on the surface of the nylon cloth so that the debris on the nylon entered the bottle. Preparation of types of microplastics in underground river water samples in Caves used solvents that have been diluted, namely 30% H_2SO_4 and 30% H_2O_2. Then, 20 mL of 30% Hydrogen sulfate and 30% Hydrogen peroxide solution were added to the sample into a glass bottle and closed. Water samples submerged in the diluent solution and incubated at room temperature for 24 hours.

Sediment samples were taken from Lowo Cave, Banyu Cave and Sengik Cave using 250 g glass bottles and then packed to the laboratory. Sediment samples were dried in an oven at 100°C for 8 hours until it dry. The dry samples were weighed 50 g each and dissolved in 300 mL of concentrated NaCl. The samples were then stirred and allowed to stand until suspended. The sediment suspension consists of supernatant and pellets. The supernatant was separated from the pellet and then filtered using a 200 nm nylon cloth. The filtering was carried out two times until the microplastic particles were collected. Next, 20 mL of 30% H_2O_2 was added with a ratio of 1:3 and stored again for 24 hours. Finally, the supernatant solution was filtered again using filter paper and then rinsed using 300 mL of distilled water.

Sample Identification

Microplastics were visually observed and identified using a trinocular stereo microscope connected to Opti Lab, with Opti Lab software and Image Raster 3.0, to determine the abundance, type, and colors.

All particles suspected to be microplastics were then separated from the samples using precision tweezers and classified into five categories: fragments, fibres, foam, films and granules. In this study, no foam and granule-type microplastics were found.

Microplastic color identification was done visually by grouping colors: blue, white, red, yellow, black, green, pink and brown.

Data Analysis

Data were analyzed descriptively, including microplastics abundance, type and color. The results of data analysis are displayed in the form of tables and graphs for each sample at each location. Sources of microplastic pollution were identified based on environmental conditions around the Cave, which were related to microplastic pollution in the water and sediment in the Cave.

The analysis results would be compared with the literature based on the types of microplastics found, so that, they could explain the environmental conditions that may influence microplastic pollution in the Caves. Calculation of microplastic abundance was calculated using equation $N = n/v$, where N is the microplastic abundance (particles/liter), n is the microplastic (particles) count and, v is the volume of the water sample (liter) (16). Later, microplastic particle concentrations were compared across sampling sites by abundance, types and colors using the Kruskal-Wallis test, as the data wasn't normally distributed (17-18).

RESULTS

The analysis was carried out by comparing three southern Malang Regency caves: Lowo Cave, Banyu Cave and Sengik Cave. The comparison of the three caves is based on the fact that research related to microplastics in caves is rarely carried out in Indonesia, and the local community often uses the water sources in the caves. The number of residents around the Cave, which is rarely found, can influence the number of microplastic particles. In Sengik Cave the population is far from the Cave, but using underground river water with interconnected pipes as a source of life for the community allows the local community to carry out activities around Sengik Cave frequently; the abundance obtained was 2.3 particles/L. Meanwhile, in Banyu Cave, the lowest abundance of microplastic particles in the water was 1.9 particles/L; this happened because Banyu Cave is in the middle of forests and hills.

Table 1. Summary of the Average Abundance of Microplastics in Karst Areas in Various Studies

Research Location	Sample	Average Abundance of Microplastics	Reference
Karst Plateau of Guizhou province, China	Soil	3150 particles/Kg	(24)
Yulong River, Guilin, China	Water	4 particles /L	(27)
	Sediment	247–1708 particles /Kg	
Cliff Cave, Missouri, United States	Water	7.1 ± 2.1 particles /L	(44)
	Sediment	842.7 ± 166.4 particles /Kg	
The Gulf of Orosei (Sardinia, Italy)	Sediment	17.2 ± 7.7 particles /Kg	(28)
Samcheok and Donghae, Korea	Water	0.042–1.026 articles/L	(26)
Ţarina and Josani spring, Romania	Water	0.05 particles /L	(45)
Kamniška Bistrica, Slovenia	Water	59 particles /L	(25)
	Sediment	22 particles /Kg	
The Bossea karst system, Italy	Water	28 particles /L	(46)
Wujiang river basin, China	Sediment	1354 particles /Kg	(47)
Shiraz watershed, Iran	Water	0.1 to 1.3 particles /L	(48)

Abundance of Microplastic Particles in Caves

This study found that the highest abundance of microplastics in water samples was in Sengik Cave at 2.3 particles per liter, while the lowest was 1.8 particles per liter at Banyu Cave. In this study, the highest pofusion in water is relatively low compared with other studies, even though not the least.

In sediment samples, the highest abundance of microplastics was 2 particles/Kg, while the lowest was 0.6 particles/Kg.

A graph of the abundance of microplastics in underground river flows in caves in the South Malang karst area can be seen in Figure 1 below:

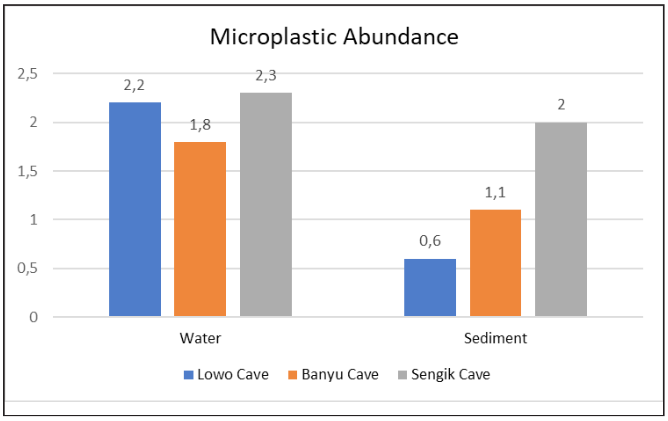

Figure 1. The Abundance of Microplastics in the Underground River Flows in the Caves of the Malang Selatan Karst Region

Types of Microplastic Particles in Caves

The number of microplastic particles of each type in the water and sediment from each Cave can be seen in Table 2 below.

Table 2. Types of Microplastic Particles in Caves

Types of Microplastic	Lowo Cave		Banyu Cave		Sengik Cave	
	W	S	W	S	W	S
Film	1	0	0	2	0	0
Fragment	0	1	0	0	0	0
Fiber	10.5	3	9	4.5	11.5	2.5

W: water sample
S: Sediment sample

Fiber-type microplastics were the dominant form found in both water and sediment samples at all locations. This dominance was likely due to the high number of fibers discovered (41 particles) in both water samples and sediment samples. Meanwhile, only one particle was found from the Cave Lowo River sediment sample fragment type. Three types of microplastic film were found: one particle from a water sample from the Lowo Cave River and two particles from a sediment sample from the Banyu Cave River.

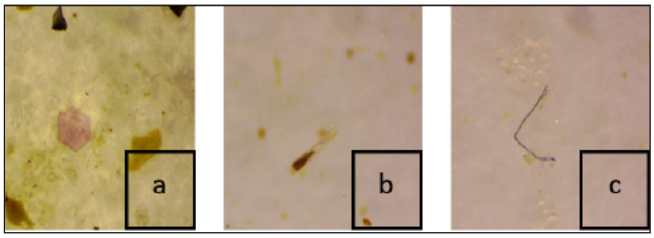

Figure 2. The Types of Microplastic Particles Obtained are: (a) Fragments; (b) Film; (c) Fibers.

Microplastic fragments come from more considerable plastic materials, such as bags, bottles, containers and food products. Exposure to sunlight, temperature, ocean waves, or human activity, such as mechanical crushing, can cause this fragmentation process. Fragmentation can occur in open environments or locations such as waste processing and disposal facilities (19). Meanwhile, this type of film comes from thin plastic products, such as plastic shopping bags, food wrappers, consumer product wrappers, and other thin plastic materials. The degradation or fragmentation process of these products can produce microplastic films. Meanwhile, fibre microplastics have long and thin fibres. Despite their tiny size, these fibres retain their thin, elongated shape, differentiating them from other microplastics. This type of microplastic usually comes from synthetic clothing (polyester or nylon), textile products, carpets, and plastic products that contain fibre (20). While Malang Regency is known for its tea plantations, the diverse landscape in southern Malang regencies offers opportunities for sugarcane, horticulture, coffee, chocolate, and teak forests in the southern limestone mountains (21).

Colors of Microplastic Particles in Caves

The colors of microplastics found in the three caves were blue, white, red, yellow, black, green, pink and brown. Blue is the dominant color (42%) in microplastics found in water and sediment in underground rivers in caves followed by red (29%) and black (13%).

The color of microplastics varies greatly depending on the color of the original plastic being decomposed. Microplastic particles can have a wide variety of colors, including transparent or clear, depending on the properties of the original plastic. For example, polyethylene plastic may tend to be more transparent or white, while polypropylene may have brighter or more colorful colors (19,22). Since the southern part of Malang Regency is dominated by sugarcane and horticulture, there's a risk of plastic bag use during planting, growing, and harvesting (23). The colors of microplastics in this study are depicted in Figure 3.

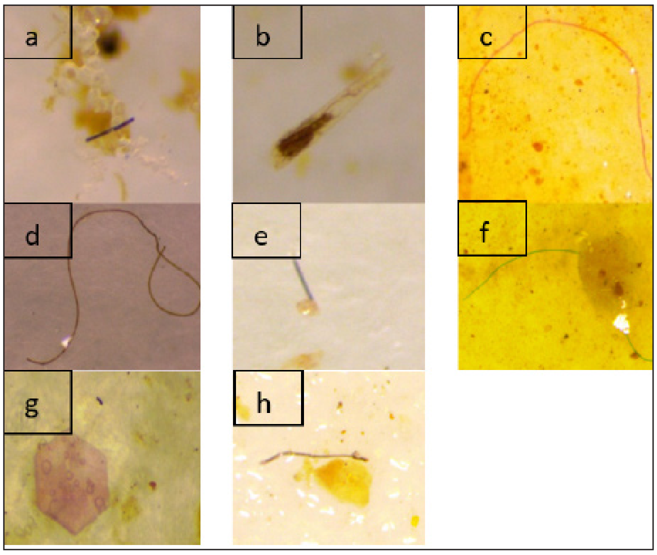

Figure 3. The Colors of Microplastics Found in Three Caves. (a) Blue; (b) White; (c) Red; (d) Yellow; (e) Black; (f) Green; (g) Pink; (h) Brown

Using Kruskal-Wallis analysis, it was found that there were no significant differences (p>0,05) in the abundance, type and color of microplastics in Lowo Cave, Bayu Cave and Senik Cave with values (p) of 0.368, 1.000, and 0.964 respectively.

DISCUSSION

Analysis of ten publications reveals that research on microplastics in karst areas suggest that minimal average microplastic present in underground river water and sediment. In contrast, significantly higher levels have been reported in surface river samples from karst areas. Additionally, soil samples from karst areas have been found to be highly contaminated with microplastics (24). One of the reasons for the high content of microplastics in the soil is caused by plastic waste that is carelessly thrown away on land so that it can undergo mechanical degradation into microplastics through a physical breakdown process due to sunlight, water and other activities.

Based on Table 1, we can conclude that the highest average abundance of microplastics in water samples from karst areas is in Kamniška Bistrica, Slovenia, with an abundance of 59 particles/L (25), while the lowest abundance of microplastics is in the Samcheok and Donghae areas, Korea, with an abundance of 0.042 particles/L (26). Then, in river sediments in karst areas, the highest abundance is in the Yulong River area, Guilin, China, with an abundance of 1708 particles/Kg (27), and the lowest is in The Gulf of Orosei Sardinia, Italy with an abundance of 17.2 particles/Kg (28). The difference in microplastic concentrations in water and sediment appears to be lower in river water than in sediment; this involves deposition, capture events, and interactions with various elements in the river ecosystem.

Microplastic particles floating in water can be deposited and captured by sediment at the bottom of the river. This process can cause the accumulation of microplastics in river sediment layers, ultimately resulting in higher concentrations than those found in the water. Water currents can also influence the movement of microplastics in rivers. Some microplastic particles can remain in the water, while others may be more likely to settle at the bottom of rivers. Water currents can cause concentration differences between sediment and water. Besides, fibres and beads type microplastic can go trough to the stream of water and riddled, hence the minor size of particles can be discharged and suspended in water (29). With smaller size floating, this process makes microplastic more difficult to detect in water than sediment, since smaller particles can become trapped and accumulated.

Karst soil is an effective filter medium and can reduce the number of microplastic over its pipeline holes, so it lowers the amount that reach underground water. Moreover, the abyss and crevice in Karst area become pathways for surface water to percolate into karst system and form underground stream (30). In addition, larger microplastic particles can be clogged or trapped in the soil layer, while filtered water is cleaner. The results of the abundance of microplastics in sediment in this study are meagre compared to studies in other areas, this could be because there is few human activities where the source of plastic pollution originates. Plastic pollution is often related to human activities, such as industrial waste, urban waste, or coastal activities. In addition, the presence of surface rivers or water channels that enter the Cave directly is limited so that the presence of microplastics in the sediment is minimal, reducing the possibility of microplastics entering the Cave from the outside environment. Microplastics can often be part of the food chain in aquatic ecosystems (31). If caves have low populations of plankton-eating organisms or a lack of food chains involving microplastics, this could contribute to the low abundance of microplastics in cave sediments.

It is so important to understanding the physical and chemical characteristics of the environment to insight the movement of microplastics in the environment. Physical characteristics such as soil sediment type, granulometry, composition of organic matters in the soil, and local hydrodynamics play an important role in the fate and transport of microplastics. Fine sediment can affect microplastics more effectively than soil that has a coarse texture, besides that strong currents in the water can also influence the process and length of time that microplastics form, thereby affecting local microplastic concentrations (32). This could be an indication of why there are fewer microplastics in underground rivers in karst areas than in others. Apart from that, further research needs to be carried out to determine the abundance and types of microplastics in each layer of soil and rock, because their distribution can reveal further what and how to prevent further pollution (33).

The discovery of a much smaller distribution and abundance of microplastics in this research area could occur due to the limited number of research samples, because not all underground rivers have been exposed and explored so sampling may not be representative enough. This unexpected finding can certainly be a catalyst for further research both quantitatively and qualitatively using various perspectives. The uniqueness of the karst area here poses a particular challenge for microplastic research, because the limited vegetation, which according to (34), makes the abundance of microplastics greater in the soil due to the lack of natural filters on the surface.

Microplastic particles of the fibre type dominate in the three caves studied. This type of fibre usually comes from cloth and nylon fibres; in water, this type of fibre can be assumed to come from waste water from washing clothes and flying dust. In sediment, the fibre type dominates due to the accumulation of water in the sediment, so there are microplastic deposits in the sediment. Fragments were found in the sediment in Lowo Cave because plastic bottles were found floating on the surface of the water in the Cave, making it possible that the plastic bottles had underwent fragmentation due to the impact between the water and the cave rock, by research that microplastic particles can be formed from the impact of currents and waves (35).

Synthetic microfibers shed from textiles, clothing, and industrial processes are primary suspects, their persistence and potential to absorb harmful chemicals posing a significant threat. However, natural cellulose fibres from vegetation also contribute to the fibrous tapestry, raising questions about their role in altering sediment dynamics and microbial communities. Identifying the specific culprits demands advanced analytical techniques, allowing us to differentiate between synthetic and natural sources and gauge the true extent of microplastic pollution (36).

The potential damage does not only impact nearby and open ecosystems. The presence of microplastics, especially synthetic fibers in sediments raises concerns regarding bioaccumulation in the food chain (37). These tiny threads can be mistaken for food by filter feeders and shellfish, eventually finding their way onto our dinner plates. The potential health risks associated with the consumption of microplastics are still being investigated, but the possibility requires caution and proactive action. In other studies, the discovery of microplastics in human placentas raised concerns (14), microplastic can not only directly inhibit skin growth (38) and reduce the quantity of testosterone (39), but these small particles can act as agents for dangerous pollutants, if inhaled they have the potential to contaminate the lungs (12) and cause serious health problems.

To reduce pollution and overcome this significant challenge, a comprehensive approach is needed from various sectors and perspectives. At sources of pollution, reduction and limitation of the use of single-use plastics such as textiles containing microfibers need to be implemented strictly. Alternative solutions using plastic polymers that are easily degraded naturally and innovations in better wastewater treatment technology can provide definite hope. Massive campaigns related to public awareness can change consumer behavior to make choices towards a sustainable environment and responsible waste management. From a scientific perspective, it is very important to carry out ongoing research with activities that focus on finding concentrations and distribution patterns of microplastic contamination, as well as factors that influence the ecological impact. International collaborations can share knowledge and accelerate the development of mitigation strategies. Policy interventions are equally crucial, stricter regulations on plastic production and disposal, coupled with extended producer responsibility schemes, can incentivize the development of sustainable alternatives. Investing in green technologies for wastewater treatment and microplastic removal is essential for safeguarding our water resources (40).

The color of microplastic particles is dominated by blue and black, this indicates the amount of pollutant absorbed by the microplastic particles. It is also often explained in general that a dark color means it has not experienced significant discoloring (41). In water and sediment, colors that appear transparent, such as yellow and white, were also found; this could be due to the water and sediment being exposed to water and sediment for a long time.

Blue pigments, particularly certain phthalates and azo dyes, are known for their high resistance to degradation. This inherent resilience could lead to their enrichment in the environment compared to other colors, which might degrade faster. Perhaps blue-colored plastics are more prevalent in specific industries or applications relevant to the karst area. For instance, agricultural tarpaulins or blue-dyed textiles used in tourism or mining activities could be significant contributors (42). The unique hydrological characteristics of karst systems, with their underground networks and sinkholes, could influence the transport and fate of microplastics differently based on their colors. Blue pigments might have specific properties, such as lower buoyancy or higher affinity for certain karst rock types, leading to their preferential accumulation in specific locations. While the blue dominance is intriguing, it is crucial to remember that it represents just one facet of the microplastic problem in karst areas.

Other colors and types of microplastics undoubtedly exist, each posing its own unique set of ecological threats. Focusing solely on blue could lead us to overlook other potentially harmful microplastics, hindering effective mitigation strategies. Conducting long-term monitoring programs to track changes in microplastic color composition over time is crucial (43). Investigating the specific sources and transport dynamics of blue microplastics can inform targeted interventions and source reduction strategies. Collaborating with geologists, hydrologists, and social scientists can help us unravel the complex interplay between environment, human activity, and microplastic pollution in karst areas.

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CONCLUSION

Despite varying levels of microplastic contamination across three South Malang caves, our findings expose a worrying truth: karst soil, often considered a natural filter, is not immune to plastic pollution. While trace amounts of microplastic particles were detected, their presence implies a significantly higher degree of surface contamination than previously assumed. This highlights the understudied vulnerability of karst ecosystems and the urgent need for further research on microplastic polymers in underground rivers.

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