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The first evidence of microplastics in plant-formed fresh-water micro-ecosystems: *Dipsacus* teasel phytotelmata in Slovakia contaminated with MPs

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Abstract
Tiny pieces of plastic, or microplastics, are one of the emerging pollutants in a wide range of different ecosystems. However, they have, thus far, not been confirmed from phytotelmata – specific small water-filled cavities provided by terrestrial plants. The authors confirmed microplastics (141 μm – 2.4 mm long fibres of several colour and blue and orange fragments with diameters of 9–81 μm) in quantities from 101 to 409 per ml in *Dipsacus* telmata from two different periods. The phytotelmata, therefore, appear to be possible indicators of current and future microplastic pollution of the environment. However, further research is needed to obtain accurate information and verify the methodology for possible assessment of the local environmental burden of microplastics.

Keywords
plants, plastics, transport, telmata

Introduction

Microplastics (MPs) are becoming an important problem (e.g. Andrady 2011; Cole et al. 2011; Weber et al. 2021 etc.). They have been recorded in a wide range of different ecosystems, from terrestrial to aquatic (e.g. de Souza Machado et al. 2018; Weber et al. 2021; Yang et al. 2021) and even in food, bottled drinking water and the organs of various organisms, including humans (e.g. Carbery et al. 2018; Jin et al. 2021;

Ragusa et al. 2021). Most studies of MPs, or SAMPs (atmospheric MPs), are more focused on the marine and freshwater ecosystems (e.g. Panebianco et al. 2019; Weber et al. 2021; Yang et al. 2021) and we still do not have enough information about their impact on organisms (e.g. Al-Jaibachi et al. 2019).

To the authors' knowledge, the presence of MPs has not yet been confirmed in phytotelmata, a wide range of generally non-permanent aquatic microecosystems in plants (e.g. Kitching 2000; Kanašová et al. 2020). Amongst the few phytotelmata in the temperate zone of Europe are dendrotelmata and phytotelmata provided by the teasel *Dipsacus* (e.g. Williams 1996, 2006; Kitching 2000; Oboňa et al. 2011; Oboňa and Svitok 2012; Kanašová 2017; Kanašová et al. 2020). Teasel phytotelmata (Fig. 1) are a relatively common, but overlooked aquatic microcosm with a very short-term occurrence of only 3 to 4 months (Kanašová et al. 2020). *Dipsacus* teasel has characteristic opposite leaves that grow on the stem above each other in several levels (the oldest near the soil surface and the youngest are the highest), clasping the stem and forming cup-like structures that collect water (water axil or telmata).

The main purpose of the sampling was to describe the seasonal dynamics of organisms living in teasel telmata. The detection of MPs in these samples was accidental and unexpected. The objective of this paper is to describe the first documented evidence of MPs in phytotelmata.

Materials and methods

Water samples with sediment from phytotelmata on teasel *Dipsacus* came from two areas of eastern Slovakia (see Map. 1) near the villages of Demjata (49°6'58.578231"N, 21°18'47.3838982"E, Fig. 2) and Kapušany (49°3'12.6212568"N, 21°20'16.680325"E).

The samples were obtained from five plants at each of two sampling localities at the end of each of five 14-day long collection periods from all levels of leaf axils at examined plants. The collection was carried out using standard methods (see Kanašová et al. 2020) using sterile containers. Therefore, contamination of the samples from another source is clearly excluded. These 50 sampled *Dipsacus* individuals provided 171 functioning phytotelmata. Altogether, 4596 ml of water and sediments were analysed (see Table 1).

In the laboratory, the samples were examined using a microscope method after transfer to a sterile Petri dish. After first MP evidence, the examination was conducted following the microscopic method (see Yang et al. 2021). Positive samples were separated and MPs photographed and measured. From positive samples, we analysed 3 ml of the total sample volume. For the greatest possible accuracy, we analysed this volume in increments of 0.5 ml, always after thorough mixing of the liquid. Quantitative data were then converted to 1 ml of sample. These examinations and measurements were conducted using a Leica M205MC stereomicroscope (magnification of 7.8–160×), equipped with a Leica DFC295 digital camera. The minimal size of particles captured and measured by this method and equipment used is 1 μm.

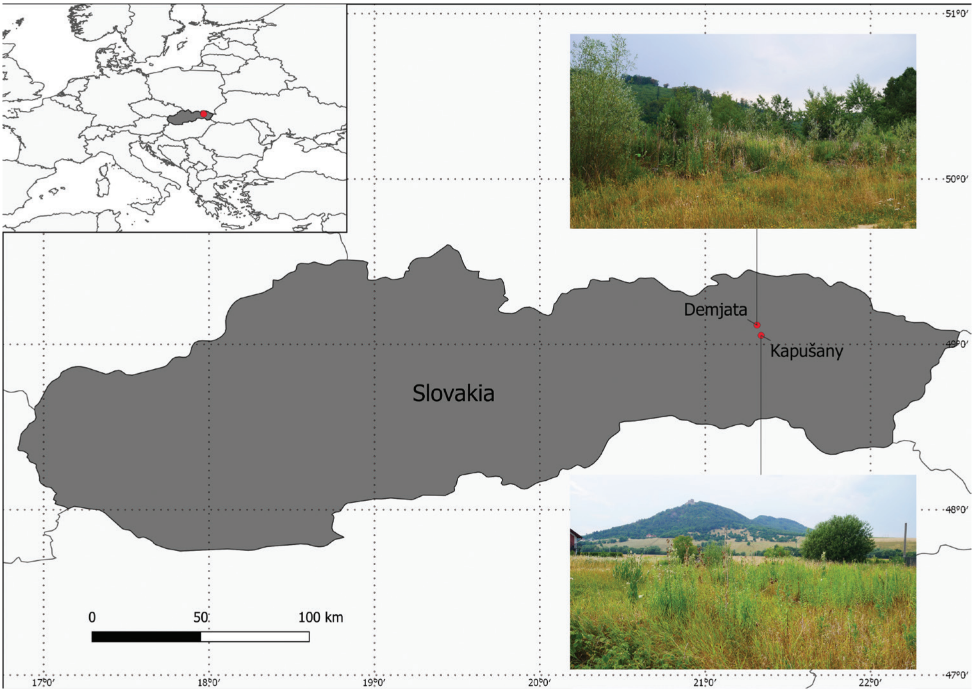
Table 1. Overview of sample volumes of individual phytotelmata.

Date	Plant number	Locality	total levels on the plant	level 1		level 2		level 3		level 4		level 5		level 6		level 7		level 8		level 9		level 10	
				sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)
6/14/ 2021	1	Demjata	6	damaged		damaged		1	38	2	29	3	17	empty									
	2	Demjata	6	1	12	2	14	3	21	empty		empty		empty									
	3	Demjata	6	damaged		1	70	2	37	3	13	empty		empty									
	4	Demjata	7	damaged		1	37	2	85	3	17	empty		empty		empty							
	5	Demjata	7	1	15	2	8	3	16	4	35	5	10	empty		empty							
	1	Kapušany	6	1	12.5	2	44	3	42	4	49	empty		empty									
	2	Kapušany	7	1	17	2	16	3	26	4	6	empty		empty		empty							
	3	Kapušany	6	1	15	2	31	3	10	empty		empty		empty									
	4	Kapušany	6	damaged		1	4	2	16	3	7.5	4	3	empty									
	5	Kapušany	7	damaged		damaged		1	35	2	4	empty		empty		empty							
6/28/ 2021	1	Demjata	8	damaged		damaged		damaged		1	35	2	18	3	14	4	3	empty					
	2	Demjata	9	damaged		1	20	2	73	3	61	4	49	5	27	empty		empty		empty			
	3	Demjata	9	damaged		damaged		1	6	2	76	3	61	4	47	5	11	empty		empty			
	4	Demjata	9	damaged		damaged		1	10	2	106	3	92	4	43	empty		empty		empty			
	5	Demjata	9	damaged		1	5	damaged		2	41	3	20	4	12	empty		empty		empty			
	1	Kapušany	8	damaged		1	5	2	51	3	41	4	60	5	45	6	20	7	15				
	2	Kapušany	9	damaged		damaged		damaged		1	42	2	37	3	26	4	25	5	5	empty			
	3	Kapušany	8	damaged		damaged		1	29	2	42	3	80	4	34	5	29	6	6				
	4	Kapušany	8	damaged		damaged		1	51	2	92	3	45	4	45	5	36	6	17				
	5	Kapušany	9	damaged		damaged		damaged		1	5	2	85	3	70	4	15	5	16	6	8		
7/12/ 2021	1	Demjata	9	damaged		damaged		1	50	2	36	3	20	4	10	empty		damaged		damaged			
	2	Demjata	9	damaged		damaged		1	9	2	6	3	17	4	29	5	12	damaged		damaged			
	3	Demjata	8	damaged		damaged		1	7	2	10	3*	11*	empty		empty		damaged					
	4	Demjata	8	damaged		damaged		1	20	2	16	3	17	4	5	damaged		damaged					
	5	Demjata	9	damaged		damaged		1	20	2	60	3	25	4	14	5*	6*	empty		damaged			
	1	Kapušany	8	damaged		1*	9*	2	76	3	95	4	83	damaged		5	12	6	3				
	2	Kapušany	7	damaged		1	12	2	17	3	7	empty		empty		empty							
	3	Kapušany	7	damaged		damaged		damaged		1	7	2	5	empty		empty							
	4	Kapušany	8	damaged		damaged		1	32	2	47	3	24	damaged		empty		damaged					
	5	Kapušany	8	damaged		1	2	2	18	damaged		3	41	4	49	5	15	empty					

Date	Plant number	Locality	total levels on the plant	level 1		level 2		level 3		level 4		level 5		level 6		level 7		level 8		level 9		level 10	
				sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)	sample number	volume (ml)
7/26/ 2021	1	Demjata	7	damaged		damaged		1*	8*	empty		empty		damaged		damaged							
	2	Demjata	8	damaged		empty		1	9	2	2	empty		empty		empty		damaged					
	3	Demjata	8	damaged		damaged		empty		1	10	empty		empty		empty		damaged					
	4	Demjata	8	damaged		damaged		damaged		1	12	2	5	damaged		damaged		damaged					
	5	Demjata	8	damaged		damaged		1	11	empty		empty		empty		damaged		damaged					
	1	Kapušany	8	damaged		damaged		1	10	damaged		2*	40*	empty		3	5	empty					
	2	Kapušany	9	damaged		damaged		1	6	2	41	3	110	4	58	5	12	6	6	damaged			
	3	Kapušany	10	damaged		damaged		damaged		damaged		1	15	2	12	empty		empty		damaged		damaged	
	4	Kapušany	9	damaged		damaged		1	5	2	55	3	55	4*	75*	5	11	empty		damaged			
	5	Kapušany	8	damaged		damaged		damaged		damaged		1	90	empty		2	9	empty					
8/9/ 2021	1	Demjata	8	damaged		damaged		damaged		1	5	2	6	empty		empty		empty					
	2	Demjata	7	damaged		damaged		1	8	2	5	3	3	empty		empty							
	3	Demjata	8	damaged		damaged		damaged		damaged		1	3	empty		empty		empty					
	4	Demjata	7	damaged		damaged		damaged		1	3	2	2	empty		empty							
	5	Demjata	8	damaged		damaged		damaged		damaged		1	5	empty		empty		empty					
	1	Kapušany	8	damaged		damaged		damaged		damaged		damaged		damaged		1	15	empty					
	2	Kapušany	8	damaged		damaged		damaged		1	47	2	6	3	14	4	2	empty					
	3	Kapušany	8	damaged		damaged		damaged		damaged		1	24	2	45	3	12	empty					
	4	Kapušany	7	damaged		damaged		damaged		damaged		1	10	empty		empty							
	5	Kapušany	7	damaged		damaged		damaged		1	36	empty		empty		empty							

Table legend: Plant number represent the serial number of the plant at the location (5 plants were examined at each location); levels on the plant represents a number of oppositely growing leaves that are able to retain rainwater; volume (ml) means the total volume of water that was captured by the opposite leaves; sample number - sample designation, * positive sample; empty cells - undeveloped phytotelma

Figure 1. Phytotelmata in the teasel *Dipsacus*.



Map 1. Location of the study areas.

Results and discussion

Overall, MPs were detected in only in 6 of 171 examined samples (incidence 3.5%). MPs consisted, in particular, of blue, black, red and white 141 µm to 2.4 mm long fibres and blue and orange fragments with diameters of 9 to 81 µm. There were 101 to 409 MPs in each positive sample (Table 2). Positive telmata were recorded only during two sampling periods (29.6.–12.7.2021 and 13.–26.7.2021) at different levels and always at both locations. These results are the first confirmation of evidence of MPs in phytotelmata on *Dipsacus* teasel (see Fig. 3).

These phytotelmata are very small and have a short lifespan (e.g. Kanašová et al. 2020). The question is, therefore, how were they polluted with MPs? The most probable contamination source is suspended atmospheric SAMPs. Fibres (Liu et al. 2019; Wright et al. 2020) and fragments (Allen et al. 2019) are the most prevalent shapes in SAMPs samples and they also dominated in phytotelmata. Our findings also support the idea that SAMPs could have an MP pollution source (Alfonso et al. 2021), whereas other paths for the spread of fibres and fragments into above-ground phytotelmata are unlikely to be possible. In the case of SAMPs' contamination, the low number of positive phytotelmata may be explained by the density of the surrounding vegetation and by the position and orientation of the water-filled cavities on *Dipsacus*.

Figure 2. Locality of the teasels in Demjata.

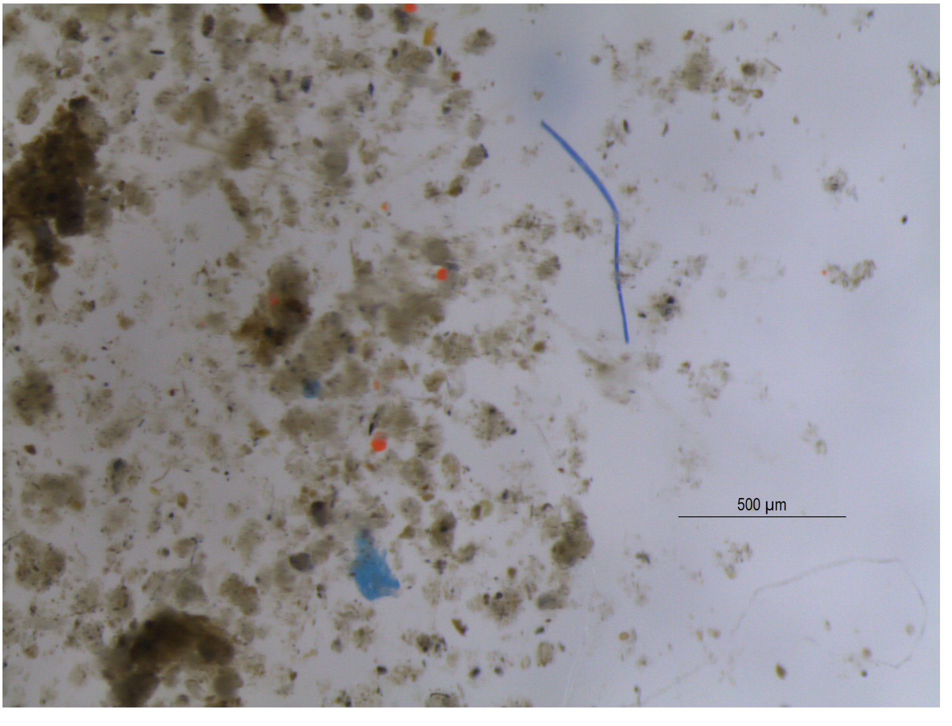


Figure 3. Microplastic fibre and fragments from a phytotelmata sample.

Table 2. The detailed information about MPs in positive phytotelmata.

Date	Plant number	Locality	Total levels on the plant	Level	Sample number	Volume (ml)	Fibers							Fragments					Average amount of MPs per 1 ml		
							Total Number (3 ml)	Colour				Min. length (mm)	Max. length (mm)	Total Number (3 ml)	Colour		Min. size (mm)	Max. size (mm)	Fibres	Fragments	Total
								Blue	Black	Red	White				Blue	Orange					
7/12/2021	3	Demjata	8	5	3	11	8	3	3	1	0	0.2051	1.5790	98	58	40	0.0107	0.0718	2.7	32.7	275.19
7/12/2021	5	Demjata	9	7	5	6	4	2	2	0	0	0.1558	1.3092	159	45	114	0.0096	0.0695	1.3	53.0	408.87
7/12/2021	1	Kapušany	8	2	1	9	12	6	3	0	3	0.1955	2.1730	22	15	7	0.0122	0.0420	4.0	7.3	101.75
7/26/2021	1	Demjata	7	3	1	8	3	3	0	0	0	0.1414	0.8832	142	28	114	0.0096	0.0528	1.0	47.3	358.42
7/26/2021	1	Kapušany	8	5	2	40	13	8	2	3	0	0.1663	2.3937	59	33	26	0.0115	0.0808	4.3	19.7	225.65
7/26/2021	4	Kapušany	9	6	4	75	0	0	0	0	0	0	0	56	18	38	0.0088	0.0551	0.0	18.7	224.73

Figure 4. The presence of snail (*Cepaea*) on teasel leaves.

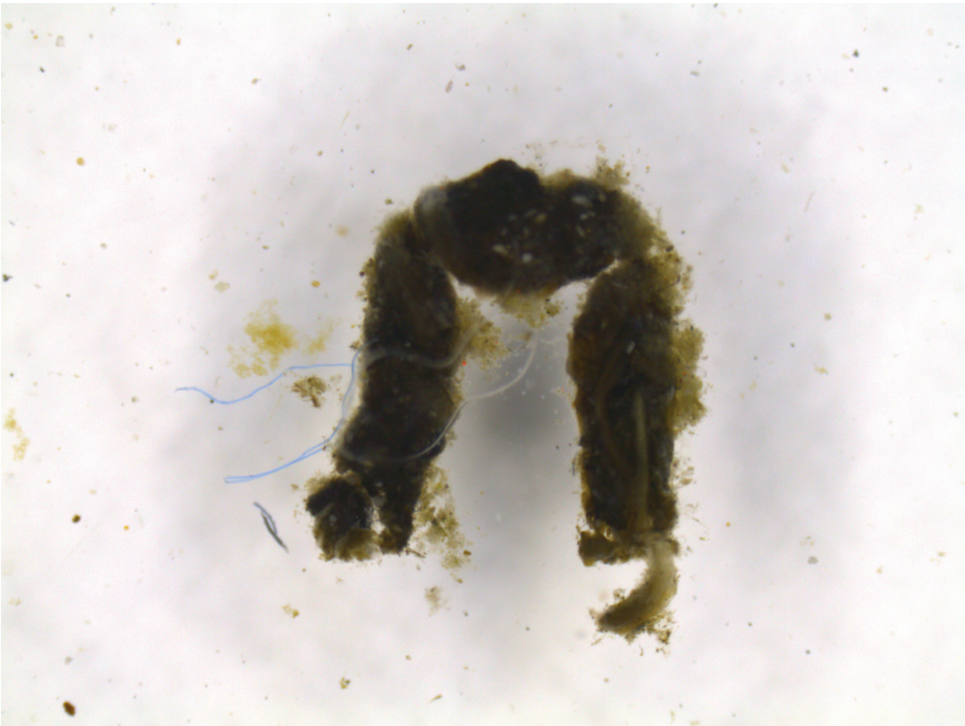


Figure 5. Microparticles in snail excrement.

The second possible pathway of contamination is zoonotic transport (active or passive) through snails (Fig. 4). Snails could transfer particles of MPs on or in their bodies (e.g. Panebianco et al. 2019). This theory can be supported by the frequent presence of living or drowned snails and their excrements in teasel phytotelma (see Fig. 5). Transmission by molluscs from soil and plant surfaces would indicate pollution from the earth's surface. In any case, the surface of the landscape, soil and vegetation could only be contaminated by the atmosphere (SAMPs) at the sites examined in this study, as no other sources of contamination are present at the localities or in their immediate surroundings.

Based on these results, aims in our future research will be: (1) to find out whether the pathway of pollution (i.e. wind transport, active zoonotic transport, passive zoonotic transport) would influence the utility of phytotelmata as indicators of microplastic pollution and (2) to test the hypothesis that the amount of microplastics in phytotelmata reflects their amount in the environment (i.e. more MPs in the environment mean more MPs in phytotelmata). Teasel phytotelmata are a relatively common, but overlooked aquatic microcosms (Kanašová et al. 2020). Due to their abundance and theoretical ability to capture MPs in several ways from the environment, they could be a good indicator of MPs occurrence (rather than directly measuring the environment). Moreover, the temporal character of phytotelmata and the succession of individual levels serves as a natural "time-lapse" sampling with the possibility of identifying temporal differences in the intensity of contamination during the growing season.

MPs have become one of the emerging pollutants in a wide range of different ecosystems (e.g. de Souza Machado et al. 2018; Carbery et al. 2018; Yang et al. 2021; Weber et al. 2021; Jin et al. 2021; Ragusa et al. 2021). The occurrence of MPs has continued to expand on a global scale and has attracted widespread attention from scientists, policy-makers and the public (e.g. Jin et al. 2021). One of the basic prerequisites for a solution and remediation is an understanding of the external forces that drive the transport and diffusion of these pollutants. Our findings point to the possibility of using phytotelmata (and/or artificial telmata) to determine the contamination of the environment by MPs and the relatively simple detection of seasonal/temporal changes in the atmospheric load of the studied sites by SAMPs. In any case, this topic and the bio-indicative potential of telmata in the environmental burden of MP assessment deserve further research and more attention.

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